# CANADIAN THESES ON MICROFICHE

# THÈSES CANADIENNES SUR MICROFICHE

National Library of Canada
Collections Development Branch

Canadian Theses on
Microfiche Service

Ottawa, Canada
K1A 0N4

Bibliothèque nationale du Canada
Direction du développement des collections

Service des thèses canadiennes
sur microfiche

## NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30. Please read the authorization forms which accompany this thesis.

## AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30. Veuillez prendre connaissance des formules d'autorisation qui accompagnent cette thèse.

THIS DISSERTATION
HAS BEEN MICROFILMED
EXACTLY AS RECEIVED

LA THÈSE A ÉTÉ
MICROFILMÉE TELLE QUE
NOUS L'AVONS REÇUE

Canada

NL 339 (r. 88/09)

THE UNIVERSITY OF ALBERTA

# Studies on the Agglutinins of *Artocarpus altilis* and *Ficus deltoidea*

by

John Bradley Hunter

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF Master of Science

IN

Pharmaceutical Sciences (Pharmaceutical Chemistry)

Faculty of Pharmacy and Pharmaceutical Sciences

EDMONTON, ALBERTA

Fall 1986

Permission has been granted to the National Library of Canada to microfilm this thesis and to lend or sell copies of the film.

The author (copyright owner) has reserved other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without his/her written permission.

L'autorisation a été accordée à la Bibliothèque nationale du Canada de microfilmer cette thèse et de prêter ou de vendre des exemplaires du film.

L'auteur (titulaire du droit d'auteur) se réserve les autres droits de publication; ni la thèse ni de longs extraits de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation écrite.

ISBN 0-315-32557-7

BIOCHEMICAL ARCHIVES
M.B.R. PRESS, INC.
P.O. BOX 111
KENYON, MINNESOTA 55946-0111

April 7, 1986

Dr. J. Brad Hunter
Faculty of Pharmacy & Pharmaceutical Sciences
The University of Alberta
Edmonton, Alberta
CANADA T6G 2N8

Dear Dr. Hunter:

Permission is granted to use a version of your manuscript, reference number A-1038, "Purification of Lectins from Artocarpus Altilis and Ficus Deltoidea by Gel Filtration Fast Protein Liquid Chromatography", by Hunter et al., in your thesis.

Sincerely yours,

Robert Derr

Robert Derr, Ph.D.
Editor

THE UNIVERSITY OF ALBERTA

**RELEASE FORM**

NAME OF AUTHOR John Bradley Hunter

TITLE OF THESIS Studies on the Agglutinins of *Artocarpus altilis* and *Ficus deltoidea*

DEGREE FOR WHICH THESIS WAS PRESENTED Master of Science

YEAR THIS DEGREE GRANTED Fall 1986

Permission is hereby granted to THE UNIVERSITY OF ALBERTA LIBRARY to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

(SIGNED) .....B. J. Hunter.....

PERMANENT ADDRESS:

.....2 Queens Court, Unit 201 W.....

.....460 Wellington St.....

.....London, Ontario N6A 3P8.....

DATED .....July 11..... 1986

"All noble things are as difficult as they are rare"

Benedict de Spinoza

1632-1677

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled Studies on the Agglutinins of *Artocarpus altilis* and *Ficus deltoidea* submitted by John Bradley Hunter in partial fulfilment of the requirements for the degree of Master of Science in Pharmaceutical Sciences.

.....

Supervisor

.....

.....

.....

.....

Date..... 9th July 1986 .....

*DEDICATED*

*To my parents, John M.W. and Marion A. Hunter,*

*for their understanding and encouragement;*

*To the memory of R.A.M.,*

*for shaping my destiny.*

## ABSTRACT

A variety of plant seeds were screened for hemagglutinating activity, immunochemical relatedness and bacterial agglutination. Extracts from the seeds of breadfruit (*Artocarpus altilis*) and jackfruit (*Artocarpus integrifolia*) exhibited a high titer non-specific for human erythrocytes. They produced immunoprecipitin reactions of partial identity with antisera directed against Osage orange *(Maclura pomifera)* lectin suggesting a structural and evolutionary relationship of Moraceae lectins.

The breadfruit lectin (BFA) was purified by salt fractionation followed by affinity chromatography on immobilized $\beta$-D-gal(1→3)$\alpha$galNAc. Gel filtration using Bio-Gel P150 suggested a molecular weight around 44,500. Electrophoresis of the purified lectin in SDS polyacrylamide gels revealed two dissimilar subunits of approximately 19,000 and 22,000 daltons. Amino acid analysis and isoelectric focusing of BFA indicated that the lectin may be acidic in nature. This lectin did not agglutinate any of a variety of bacterial species and strains. In view of its binding affinity to the Thomsen-Friedenreich (T) antigen purified BFA may prove useful in tumor binding studies.

An extract from mistletoe fig (*Ficus deltoidea*) (FDA) seeds produced non-specific precipitin reactions with human serum thus affecting immunodiffusion studies. The crude samples were also observed to agglutinate human erythrocytes and some strains of bacteria including *Chlamydia trachomatis*, a clinically significant pathogen causing a sexually transmitted disease.

Fast protein liquid chromatography (FPLC) was utilized to partially purify the lectins from crude extracts of breadfruit and mistletoe fig seeds. Comparison of the elution profiles of the affinity purified and crude extracts of breadfruit showed gel filtration FPLC can offer advantages over the traditional affinity methods in that this rapid and simple one step procedure gives a considerable purification of the lectin from the crude extract. This technique

was also used to enrich a crude FDA extract, resolving it into nine major peaks. Although every fraction agglutinated red blood cells, only the fourth fraction was active against purified elementary bodies of the bacterium *Chlamydia*. FDA may be valuable as a probe for studying bacteria, particularly clinically important pathogens such as *Chlamydia*.

## ACKNOWLEDGEMENTS

I extend sincere thanks to Dr. R.G. Micetich for his unfailing support of this project, assistance during the course of my studies, and guidance in the preparation of this thesis.

Thanks are also due to several individuals who freely contributed their advice, talents, equipment and facilities: My co-supervisors, Drs. M.R. Suresh and A.A. Noujaim; Dr. D.A. Baker, formerly of, and D. Scott Hagen of Chembiomed Ltd., whose help in the purification and characterization of the breadfruit agglutinin was invaluable; Dr. D.H. Heeley, department of biochemistry, for assistance in isoelectric focusing and two-dimensional electrophoresis; Marian Laderoute, department of immunology, for helping with electrophoresis; Dr. S.E. Jensen, department of microbiology, and Emil Dombroski, for supplying microorganisms; Tom Hantos and Dr. W.M. Wenman, Faculty of Medicine, for assisting in the Chlamydial studies; J.M. Nattriss, department of biochemistry, for conducting amino acid analysis; John Samuel, Paul Spevak and Dr. Chou Van Loc, for their support in the handling and innoculating of rabbits; Ted Germaine and Drs. R. Berzins and Y. Tam, for sacrificing the rabbits; Brij Kohli, assistant curator of vascular plants, department of botany, for simplifying botanical taxonomy; Dr. E. Keshavarz, research scientist, biotechnology department, Alberta Research Council, for FPLC separations; Dr. A.F. Almeida, for advice on carbohydrate determinations; Iche möchte Ingrid Koslowsky für ihre Hilfe danken in die Übersetzung mehrere deutsche wissenschaftche papiere; S.J.A. D'Souza for generating the computer plots; L. Dagnino, R. Keashly and P. Eu, for their patience in explaining the nuances of Textform and computer usage to me and ever willingness to help; and Ken Brown of Paper Chase Text Conversion Services Inc. for his dedication above and beyond the call of duty.

The Edmonton branch of the Canadian Red Cross Blood Transfusion Service was most generous in providing the blood used in agglutination assays. I am indebted to Chembiomed Ltd. (Edmonton, Canada) for supplying the Synsorb™, latexes and haptens used in the breadfruit agglutinin characterization.

Financial assistance in the form of a teaching assistantship from the Faculty of Pharmacy and Pharmaceutical Sciences and a studentship from the Alberta Heritage Foundation for Medical Research are gratefully acknowledged.

## TABLE OF CONTENTS


CHAPTER PAGE

I. INTRODUCTION ..... 1

A. Lectins ..... 1

1) Definition and Classification ..... 1

2) Distribution in Nature ..... 3

3) Function and Physiological Role in Plants ..... 4

4) Physicochemical Properties ..... 5

5) Biological Properties ..... 22

6) Applications ..... 23

B. Microorganisms and Lectins ..... 27

C. Botany of the Moraceae Family ..... 29

D. Aims and Objectives of Current Investigation ..... 30

E. References ..... 31

II. SCREENING OF CRUDE SEED EXTRACTS ..... 59

A. Introduction ..... 59

B. Methods ..... 59

1) Materials ..... 59

2) Preparation of crude seed extracts ..... 59

3) Screening of crude seed extracts ..... 60

4) Bacterial culturing and agglutination ..... 60

C. Results and Discussion ..... 62

D. References ..... 69

III. STUDIES ON A LECTIN FROM *ARTOCARPUS ALTILIS* SEEDS .....70
A. Introduction .....70
B. Material and Methods .....71
1) Materials .....71
2) Purification of breadfruit agglutinin .....71
3) Characterization of breadfruit agglutinin .....72
a. Latex agglutination assay .....72
b. Radioligand inhibition studies .....73
c. Gel filtration .....74
d. SDS polyacrylamide gel electrophoresis .....75
e. Isoelectric focusing in polyacrylamide gels .....75
f. Two-dimensional gel electrophoresis .....76
g. Amino acid analysis .....76
h. Carbohydrate determination .....76
i. Divalent ion dependency .....76
j. Densitometry .....77
k. Preparation of antisera in rabbits .....77
C. Results and Discussion .....78
D. References .....98
IV. PRELIMINARY INVESTIGATION OF AN AGGLUTININ FROM *FICUS DELTOIDEA* SEEDS .....101
A. Introduction .....101
B. Materials and Methods .....101
1) Materials .....101
2) Preparation of crude seed extracts .....102
3) Latex screening for affinity purification .....102
4) Fast protein liquid chromatographic purification of FDA and BFA .....102

5) Screening assays ..... 102
C. Results and Discussion ..... 103
D. References ..... 108
V. GENERAL SUMMARY AND CONCLUSIONS ..... 109
VI. APPENDIX ..... 111
VII. CURRICULUM VITAE ..... 113

## LIST OF TABLES


| Table | Description | Page |
|---|---|---|
| I-1 | Physicochemical properties of plant lectins | 7 |
| II-1 | Species examined in the current studies | 65 |
| II-2 | Hemagglutination and Ouchterlony immunodiffusion studies on extracts from a variety of plant seeds | 66 |
| II-3 | Bacterial agglutination tests with extracts from Moraceae species | 67 |
| III-1 | Distribution of breadfruit agglutinin in the various parts of the plant as determined by immunodiffusion analysis | 82 |
| III-2 | Purification protocol of breadfruit lectin | 83 |
| III-3 | Agglutination of carbohydrate coated latex beads by BFA extract | 84 |
| III-4 | Inhibition of binding of T-BSA to breadfruit lectin coated tubes by carbohydrates | 85 |
| III-5 | Carbohydrate analysis of purified breadfruit lectin using the phenol-sulfuric acid method | 86 |
| III-6 | Amino acid composition of the breadfruit lectin | 87 |
| III-7 | Comparative properties of breadfruit, jackfruit, Osage orange and peanut lectins | 88 |

## LIST OF FIGURES


| Table | Description | Page |
|---|---|---|
| II-1 | Ouchterlony immunodiffusion precipitin reactions | 68 |
| III-1 | Illustration of the breadfruit | 89 |
| III-2 | Gel filtration profile of breadfruit agglutinin on Bio-Gel P150 | 90 |
| III-3 | Standard curve for determining subunit molecular weights of breadfruit agglutinin | 95 |
| III-4 | Densitometric scan of purified BFA on reducing gel | 91 |
| III-5 | Standard curve for calculation of isoelectric point of breadfruit agglutinin | 96 |
| IV-1 | Gel filtration profile of crude and affinity purified BFA extracts on Superose 12™ | 105 |
| IV-2 | Gel filtration profile of crude extract of mistletoe fig on Superose 12™ | 105 |

## LIST OF PHOTOGRAPHIC PLATES


| Plate | Description | Page |
|---|---|---|
| III-1 | An intact breadfruit and a cross-section revealing the core | 92 |
| III-2 | Breadfruit and jackfruit seeds | 92 |
| III-3 | Ouchterlony precipitin reactions of breadfruit lectin vs antisera against *Maclura pomifera* and *Maclura pomifera* lectin vs antisera against breadfruit lectin | 93 |
| III-4 | Ouchterlony precipitin reaction of jackfruit extract vs antisera against *Maclura pomifera* | 94 |
| III-5 | SDS polyacrylamide gel electrophoresis of breadfruit lectin on a reducing gel | 95 |
| III-6 | Polyacrylamide gel isoelectric focusing of breadfruit lectin | 96 |
| III-7 | Two-dimensional electrophorectic gels of crude and purified breadfruit lectin | 97 |
| IV-1 | A branch of *Ficus deltoidea* bush showing leaves and fruit | 106 |
| IV-2 | Fruit and seeds of *Ficus deltoidea* | 106 |
| IV-3 | Ouchterlony immunodiffusion assay of *F. deltoidea* extract vs various antisera | 107 |

## LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| BFA | – | breadfruit agglutinin |
| cm | – | centimetre |
| Con A | – | Concanavalin A |
| cpm | – | counts per minute |
| °C | – | degrees Celsius |
| DTT | – | dithiothreitol |
| EDTA | – | ethylenediaminetetraacetic acid |
| FDA | – | *Ficus deltoidea* agglutinin |
| FPLC | – | fast protein liquid chromatography |
| fuc | – | fucose |
| gal | – | galactose |
| galN | – | galactosamine |
| galNAc | – | N-acetyl-galactosamine |
| glc | – | glucose |
| glcN | – | glucosamine |
| glcNAc | – | N-acetyl-glucosamine |
| g | – | gram |
| *x g* | – | times gravity |
| h | – | hour(s) |
| IgA | – | immunoglobulin A |
| IgE | – | immunoglobulin E |
| IgM | – | immunoglobulin M |
| L | – | litre |
| lact | – | lactose |
| M | – | molar |
| man | – | mannose |

| | | |
|---|---|---|
| manN | – | mannosamine |
| mg | – | milligram |
| min | – | minute |
| ml | – | millilitre |
| mm | – | millimetre |
| mM | – | millimolar |
| MPA | – | *Maclura pomifera* agglutinin |
| NA | – | neuraminidase |
| NeuNAc | – | N-acetyl-neuraminic acid |
| n.s. | – | neutral sugar |
| PAGE | – | polyacrylamide gel electrophoresis |
| PBS | – | phosphate buffered saline |
| pI | – | isoelectric point |
| PNA | – | peanut agglutinin |
| RIA | – | radioimmunoassay |
| SDS | – | sodium dodecyl sulfate |
| T antigen | – | Thomsen-Friedenreich antigen |
| TCA | – | trichloroacetic acid |
| TES | – | N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid |
| Tris | – | Tris(hydroxymethyl)aminomethane |
| uCi | – | microcurie(s) |
| ul | – | microlitre |
| um | – | micromole(s) |
| Ve | – | volume of elution |

# I. INTRODUCTION

## A. Lectins

### 1) Definition and Classification

Stillmark's discovery of a lectin in *Ricinus communis* in 1888[1] initiated research into one of nature's most interesting and complex classes of proteins. By 1975, 20 lectins had been isolated in homogeneous form[2]. Ten years later the number of plant lectin structures and specificities elucidated has risen to more than 100. This increased interest has also been reflected in growing numbers of review papers[3-18].

Lectins are studied because they act as determinants of cellular recognition and many possess interesting structural features. In addition, they are useful for the investigation of protein-carbohydrate interactions, and as reagents for the characterization and separation of cells and glycoconjugates. However, their regulation and biological role still remain a mystery[19].

Early observations of the presence of red blood cell agglutinating material in plant seeds led to the term "phytohemagglutinin" or "phytoagglutinin" or "agglutinin". The word "lectin", derived from the Latin *lectus* (past participle *legere*) meaning to choose or select, was proposed in 1954[20]. Goldstein *et al*[21] clarified this term to define **"a sugar-binding protein or glycoprotein of non-immune origin which agglutinates cells and/or precipitates glycoconjugates."** Lectins have at least two sugar-binding sites and a specificity expressed as the monosaccharide or simple oligosaccharide responsible for inhibiting lectin-induced agglutination.

Others[22,23] prefer a definition based on physicochemical properties rather than biological activities. Choosing to emphasize the interaction with carbohydrates, Kocourek and Horejsi[22] define lectins as **"sugar-binding proteins or glycoproteins of non-immune origin which are devoid of enzymatic activity towards sugars to which they bind and do not require free**

**glycosidic hydroxyl groups on these sugars for their binding."** The Nomenclature Committee of the International Union of Biochemistry (NC-IUB) dismisses this definition as being too broad and the criterion of lack of enzymatic activity difficult to apply[24,25]. Consequently, the operational definition of Goldstein *et al* is now generally accepted.

More specific terms also appear in the literature. "Protectins" was used to describe a class of invertebrate agglutinins with a protective function as protease inhibitors[26]. "Electrolectins" refer to endogenous $\beta$-D-galactoside binding lectins found in vertebrates such as chick, eel and mouse[27,28]. "Isolectins" are chromatographically distinct forms of the agglutinin similar in molecular weight, with subtle differences in amino acid composition and isoelectric point. They are not uncommon in lectins characterized to date[29-42]. A clear distinction between "isolectins" and "multiple lectins" has not been made in the literature and the terms are often used interchangeably. Isolectins are analogous to isoenzymes and represent different molecular forms of the same lectin, whereas multiple lectins are distinct and unique in their properties despite being isolated from the same source. Lectins are sometimes included under the broader classification "affinitins", a collective name for combining sites containing proteins (or glycoproteins) such as lectins, antibodies and enzymes[43-45].

In some instances, an immunochemical relationship exists between a toxin and agglutinin isolated from the same species[46]. These toxins usually have two subunits, one toxic and one sugar-binding. While similar to lectins, toxins have only one binding site and do not agglutinate cells or precipitate glycoconjugates[21,47].

Lectins resemble antibodies in that they form precipitates through specific interactions with glycoproteins and polysaccharides which are concentration dependent and may be inhibited by low molecular weight "haptens", analagous to an antigen-antibody reaction[13]. Thus, many immunological tests and procedures are applicable to lectin studies[41]. Cold agglutinins, anti-carbohydrate antibodies that precipitate at low temperatures (4°C) and can exhibit agglutinating phenomena, have been described recently[49].

The differences between antibodies and lectins are more pronounced than their similarities. Immunoglobulins are restricted to vertebrates while lectins may be found in plants and microorganisms as well as animals. Unlike lectins (constitutive), antibodies require an antigenic stimulus for their formation (induced). The immune system is flexible, allowing a variety of antibody specificities whereas lectin binding characteristics are pre-determined and restricted to carbohydrates[13]. Other differences include the metal ion requirement of some lectins for their activity unlike antibodies and the existence of isolectins as opposed to classes and subclasses of antibodies[41].

Attempts have been made to classify lectins according to their carbohydrate-binding characteristics[17,50]. This may not be entirely satisfactory since lectins exhibiting a specificity not due to simple sugars or oligosaccharides (some bacterial lectins) may be excluded[51]. In addition, some rare lectins bind amino acids[52] instead of carbohydrates. Most lectins are cytosolic although some lectins from mammalian sources are membrane bound. Hemagglutination is a general phenomenon. Antibodies and certain enzymes involved in carbohydrate metabolism could also bring about agglutination due to their polymeric nature, but are not considered lectins. The nomenclature has not yet been standardized, hence both "lectin" and "agglutinin" appear in the the literature. This is reflected in Table I-1 where the literature nomenclature is used for the specific lectins listed.

2) Distribution in Nature

Early screening studies[53-55] demonstrated the wide distribution of lectins throughout the plant kingdom. Of 5156 species tested, 16% were positive for hemagglutinating activity. Agglutinating material was present in 30% of the 852 genera evaluated[56]. A recent screen of plants from the Indian subcontinent[57] found 50 out of the 543 species tested (9%) agglutinated erythrocytes. Lectins have also been detected in commonly consumed foods[58,77,78].

Lectin research has focused on plants and plant seeds because these are inexpensive and abundantly available. However, lectins are found in many diverse taxa from bacteria to

mammals, including vertebrates[60] and mammals[61,62], invertebrates[63,64], algae[65,66], slime molds[67,68,79], bacteria[69,70], fungi[71], lichens[72], earthworms[73], fish eggs[74], and molluscs, crustaceans and gastropods[75,76].

Plant seeds generally contain the highest concentration of lectins, in the range of 2 to 10% of the total protein in most leguminous seeds[11]. Lesser amounts may also be present in stems, leaves and roots. The agglutinin content accumulates and reaches a maximum as the seed matures. Upon germination there is a progressive decline in lectin content, in parallel with the decrease of reserve protein. In mature plants lectin activity is localized within the seeds[18]. Material that cross-reacts with antiserum against the seed lectin has been found in stems and leaves but not immature seeds[80-82]. Lectin synthesis has also been detected in the apical part of adventitious roots[83].

The agglutinating activity of lectins was speculated to be under genetic control[84]. A lectin gene mutation in *Phaseolus vulgaris* resulted in reduced levels of seed lectin[85]. The chromosomes responsible for lectin synthesis in *Triticum aestivum* and *Aegilops umbellulata* have also been investigated[86].

3) Function and Physiological Role in Plants

The exact function and physiological role of lectins in plants is not clear and is the subject of much speculation. The high amounts of lectins found in Leguminosae seeds and the homologous nature of lectins of different Leguminosae tribes suggest that lectins are important to the plant[87]. Many functions have been proposed[11] including antibody-like activity against soil bacteria[18] and viruses[15,88], transport and storage of sugars[16], control of seed germination and development, regulation of plant cell extension[89], binding of symbiotic nitrogen-fixing bacteria to legumes[11,18,56,90-93], protection against insect predators[94] and fungal phytopathogens[95,96], enzymes[18,97,98] and metabolism[99], and determinants of inter- and intracellular recognition[100-102].

If lectins are necessary to protect plants against microorganisms it is difficult to explain why some plants in which lectins have not been detected are able to flourish[11], or why lectins appear to act as receptors for some bacterial pathogens[56]. The presence of lectins in stems, leaves and roots create some problems for the theory of seed germination and development. The hypothesis of plant-symbiont attachment has generated conflicting data[18]. No conclusive evidence has been presented for any of the proposed physiological roles.

The properties of lectins in themselves may suggest some unique biological roles. For example, the specificity of binding sites implies the recognition of complementary saccharide receptors with which the lectin may interact. This interaction could involve the cross-linking of glycoproteins and glycolipids as part of a structural role, if more than one binding site was present. The binding of cells evidenced by agglutination activity may promote functions such as morphogenesis and phagocytosis. The abundance of lectins suggests a structural rather than enzymatic role[103]. While it is reasonable to expect the biological role of lectins to be determined by their known biochemical properties, it may be that the biological properties have no relation to their function in nature[18].

4) Physicochemical Properties

Lectins differ chemically and structurally and exhibit dissimilar biological and physicochemical properties within the same botanical family or genus[104]. Plant lectins have molecular weights ranging from 36,000 (wheat germ agglutinin) to 250,000 (lima bean lectin) with most around 100,000 daltons. The majority are composed of two or four identical subunits. However the existence of lectins with nonidentical subunits have been reported in soybean, horse gram, lentil and garden pea. Most lectins are themselves glycoproteins with a carbohydrate content of 4 to 10%. Examples of a few lectins lacking carbohydrates are concanavalin A and peanut lectin. The amino acid composition of lectins usually shows a high amount of serine, threonine and aspartic acid with significantly low sulfur-containing amino acids. Exceptions such as wheat germ, potato and pokeweed lectins rich in cysteine, are also

known. In some instances (Con A, pea, lima bean), biological activity is dependent on the presence of divalent metal ions[18]. The diversity of physicochemical properties among lectins is comprehensively summarized in Table I-1.

Table I-1. Physicochemical properties of plant lectins characterized up to October, 1985.

| Species[1] | Family | Molecular weight (daltons) | Sub-units | Subunit size (daltons) | Carbohydrates[2] | Specificity | | Mitogenic | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Human blood group[3] | Sugar | | |
| ***Abrus precatorius*** (jequirity bean) | Leguminosae | | | | | None | D-gal | | 105-112 |
| APA | | | | | | | | | |
| Abrus | | 134,900 | 4 | 32,200<br>33,800 | | | | | |
| Abrin A | | 62,500 | 2 | | Yes (Abrin) | | | Yes (Abrin) | |
| Abrin C | | 65,600 | 2 | | | | | | |
| ***Adenia digitata*** (modecca flower) | Passifloraceae | 63,000 | 2 | 28,000<br>38,000 | | | terminal gal | | 113-118 |
| ***Aegopodium podagraria*** (ground elder) | Apiaceae | 480,000 | 8 | 55,000<br>60,000 | 11% n.s. | None | galNAc | | 119 |

[1]Nomenclature used (lectins or agglutinins) as given in the literature
[2]"Yes" indicates carbohydrate present, with amount and type undetermined
[3]"None" signifies non-specific agglutination of A,B,O and AB blood groups

| Species | Family | Molecular weight (daltons) | Sub-units | Subunit size (daltons) | Carbohydrates | Specificity: Human blood group | Specificity: Sugar | Mitogenic | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| ***Agropyrum repens*** (couch grass) | Gramineae | | | | | | | | |
| AREL (embryo) | | 36,000 | 2 | 18,000 | | None | glcNAc | | 120 |
| ARLL (leaf) | | 39,000 | 2 | 19,500 | | A | galNAc | | |
| ***Aloe arborescens*** (aloe) | Liliaceae | | | | | | | | |
| P-2 | | 18,000 | 2 | 7,500<br>10,500 | >18% | None | | Yes | 121 |
| S-1 | | 24,000 | 2 | 12,000 | >50% | (stronger than P-2) | | No | |
| ***Amphicarpaea bracteata*** (hog peanut) | Leguminosae | | | | 2% n.s. | $A_1$, $A_1B$ | galNAc | | 122 |
| ***Arachis hypogaea*** (peanut)<br>PNA | Leguminosae | 110,000 | 4 | 27,500 | 0 | T, Tk (NA treated) | $\beta$-D-gal(1→3)D-galNAc<br>D-gal | some sources | 123-133 |
| ***Artocarpus integrifolia*** (jackfruit) | Moraceae | 62,000 | 4 | 13,000<br>18,000 | 3% | None | $\alpha$-D-gal<br>galNAc | Yes | 134-143 |
| | | 39,500 | 4 | 10,000 | | | | | |

| Species | Family | Molecular weight (daltons) | Sub-units | Subunit size (daltons) | Carbohydrates | Specificity | | Mitogenic | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Human blood group | Sugar | | |
| ***Bauhinia purpurea*** (camels foot tree) | Leguminosae | 195,000 | | | 7.7% n.s.<br>3.4% glcNAc | None<br>M,N | galNAc<br>D-gal | | 144-148 |
| ***Brachypodium sylvaticum*** (false brome grass) BsyL | Gramineae | 36,000 | 2 | 18,000 | | None | glcNAc | | 149 |
| ***Butea frondosa*** (dhak or palas tree) | Leguminosae | 141,000 | | 36,000<br>65,000 | 4.2% n.s.<br>0.5% glcN | O>A=B | D-gal | | 150 |
| ***Cannabis sativa*** (hemp, marijuana) | Cannabinaceae | | | | | None (papain treated) | glc and its derivatives | | 151-152 |
| ***Canavalia ensiformis*** (jack bean) Con A | Leguminosae | 55,000<br>110,000 | 2 (pH<5.6)<br>4 (pH 5.8-7.0)<br>higher aggregates (pH>7.0) | 25,500 | 0 | None | α-D-man<br>α-D-glc | Yes (dependent on aggregation) | 153-166 |
| ***Caragana arborescens*** (Siberian pea tree) CAA | Leguminosae | 60,000 | 2 | 30,000 | Yes | None | galNAc<br>D-gal | | 167 |
| ***Cerastium tomentosum*** (Mouse ear) | Caryophyllaceae | | | | | H | glcNAc<br>salicin | | 168 |

| Species | Family | Molecular weight (daltons) | Sub-units | Subunit size (daltons) | Carbohydrates | Specificity | | Mitogenic | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Human blood group | Sugar | | |
| ***Chelidonium majus*** (greater celandine) CMA | Papaveraceae | 23,000 | 2 | 9,500 11,500 | 0 | None | glcNAc | | 169 |
| ***Cicer arietinum*** (chick or gram pea) | Leguminosae | 44,000 | 2 | 26,000 | <0.5% | None (papain treated) | IgM, fetuin no simple sugars | No | 170 |
| ***Clerodendron trichotomum*** (glory vine) CTA | Verbenaceae | 56,000 | 2 | 28,000 | Yes | Cad(+) | galNAc D-gal | | 171 |
| ***Crotalaria juncea*** (sunn hemp) | Leguminosae | 120,000 | | | 5-9.8% | None | D-gal | | 172-173 |
| ***Croton tiglium*** (purging nut) | Euphorbiaceae | 120,000 | 2 | 55,000 | 0.75% n.s. | sheep, animals (hemolytic for rabbits) | no simple sugars | | 174 |
| ***Cytisus scoparius*** (Scotch broom) | Leguminosae | | | | | | | | |
| CSI | | | | | | | | | |
| CSIa | | 70,000 | | 30,000 | | | D-gal, galNAc | | 175 |
| CSIb | | | | 30,000 | | | galNAc | | |
| CSII | | 75,000 | | 30,000 | | | galNAc | | |

| Species | Family | Molecular weight (daltons) | Sub-units | Subunit size (daltons) | Carbohydrates | Specificity: Human blood group | Specificity: Sugar | Mitogenic | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| ***Cytisus sessilifolius*** (common cytisus) CSA | Leguminosae | 110,000 | | | | O(H) | α-L-fuc<br>β-D-glcNAc(1→4)-β-D-glcNAc | | 176 |
| ***Datura stramonium*** (Jimson weed, thorn apple) | Solanaceae | 86,000 | 2 | 40,000<br>46,000 | 28-37% n.s. (mainly arabinose) 4.5% glcN, 6.3% hydroxproline residues | None | β(1→4) linked oligomers of glcNAc | Yes | 177-184 |
| ***Dolichos biflorus*** (horse gram) DBA | Leguminosae | | | | | | | | |
| A | | 113,000 | 4 | 27,300<br>27,700 | 3.8% | $A_1$ | α-D-galNAc | | 185-192 |
| B | | 109,000 | 4 | 26,000 | | | | | |
| ***Dolichos lablab*** (Indian or hyacinth bean) | Leguminosae | 110,000 | | 16,000<br>40,000 | 2% n.s. | None | glcNAc<br>methyl α-D-mannopyranoside | | 193 |
| ***Eranthis hyemalis*** (winter aconite) EHL | Ranunculaceae | 62,000 | 2 | 30,000<br>32,000 | 5% | O | galNAc | | 194 |
| ***Erythrina cristagalli*** (cockspur coral tree) ECA | Leguminosae | 56,800 | 2 | 26,000<br>28,000 | 4.5% man, fuc, xylose, glcNAc | None | lacNAc, galNAc D-gal | | 195 |

| Species | Family | Molecular weight (daltons) | Sub-units | Subunit size (daltons) | Carbohydrates | Specificity: Human blood group | Specificity: Sugar | Mitogenic | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| ***Erythrina edulis*** | Leguminosae | 56,000 | 2 | 27,000 | 7.8% n.s. | None | D-lac<br>p-nitrophenyl-β-D-galactoside | | 196 |
| ***Erythrina indica***<br>***(E. variegata)***<br>(Indian coral tree) | Leguminosae | 68,200<br><br>66,200 | 2<br><br>2 | 30,000<br>33,000<br>30,000<br>34,000 | 5.6, 9-12% n.s.<br>0.46-1.5% glcN | O>B>A | D-gal | | 197-199 |
| ***Euonymus europeus***<br>(prickwood)<br>EEA | Celastraceae | 127,000<br>166,000 | | | 4.8% D-gal<br>2.9% D-glc<br>2.8% glcNAc | B, O(H) | | | 200-201 |
| ***Euphorbia characias***<br>(Mediterranean spurge) | Euphorbiaceae | 80,000 | 2 | 40,000 | 11.3% | None | D-gal | Yes (latex)<br>No (purified lectin) | 202 |
| ***Euphorbia heterophylla***<br>(annual poinsettia)<br>EHA | Euphorbiaceae | 65,000 | 2 | 32,000 | | None | galNAc | | 203 |
| ***Glycine max***<br>***(Soja hispida)***<br>(soybean)<br>SBA | Leguminosae | 120,000 | 4 | 30,000 | 5.5-6% | None | galNAC,<br>D-gal | Yes (polymeric forms) | 204-218 |

| Species | Family | Molecular weight (daltons) | Sub-units | Subunit size (daltons) | Carbohydrates | Specificity: Human blood group | Specificity: Sugar | Mitogenic | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| ***Griffonia simplicifolia (Bandeiraea simplicifolia)*** | Leguminosae | | | | Yes (GSA-I) | | | | |
| GSA | | | | | | (GSA) B, Tk | $\alpha$-D-gal | | 219-230 |
| BS-I | | 114,000 | 4 (A,B) | | | (GS-I) B | D-glcNAc | | |
| 5 isolectins | | | | | | | | | |
| BSI-$B_4$ | | | | | | (GS-$IA_4$) A | galNAc, $\alpha$-D-gal | | |
| BSI-$AB_3$ | | | | | | (GS-$IB_4$) B | $\alpha$-D-gal, $\beta$-D-gal | | |
| BSI-$A_1B_2$ | | | | | | | | | |
| BSI-A,B | | | | | | (GS-II) Tk | glcNAc | | |
| BSI-$A_4$ | | | | | | | | | |
| BS-II | | 113,000 | 4 | 30,000 | | | | | |
| ***Hordeum vulgare*** (barley) | Gramineae | 31,000<br>36,000 | 2 | 18,000 | 0 | None | glcN, galN, manN (but not with N-acetyl derivatives) | | 231-233 |
| ***Hura crepitans (H. brasiliensis)*** (sand-box tree) 3 isolectins | Euphorbiaceae | 140,000 | | 29,000<br>31,000<br>35,500<br>37,500 | Yes | None | | Yes | 234-235 |

| Species | Family | Molecular weight (daltons) | Sub-units | Subunit size (daltons) | Carbohydrates | Specificity: Human blood group | Specificity: Sugar | Mitogenic | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| ***Iberis amara*** (candytuft) IAA | Cruciferae | | | | | M | galNAc | | 236-237 |
| ***Laburnum alpinum*** (Scotch laburnum) LAA | Leguminosae | | | | | O(H) | glcNAc N,N'-diacetylchitobiose | | 238-240 |
| ***Lactuca scariole*** (prickly lettuce) PLA | Compositae (Cichorium tribe) | 84,000 | | | Yes | mouse, rat | glycopro-teins best inhibitors | Yes | 241-242 |
| ***Lathyrus odoratus*** (sweet pea) LOA | Leguminosae | 37,000<br>42,800 | 2 | 19,000<br>4,400<br>17,000 | 0.5% n.s. | None | D-glc, D-man, their α-glycosides | Yes | 243-245 |
| ***Lathyrus sativus*** (grass pea, chickling vetch) | Leguminosae | 42,900<br>49,000 | 2 | 21,000<br>4,400<br>19,000 | 2% n.s. | None | D-glc, D-man, their α-methyl-glucosides | Yes | 246-247 |
| ***Lens culinaris (L. esculenta)*** (lentil) LcH, LcA | Leguminosae | | | | 2% | None | α-D-man, α-D-glc | | 248-253 |

| Species | Family | Molecular weight (daltons) | Sub-units | Subunit size (daltons) | Carbohydrates | Specificity Human blood group | Specificity Sugar | Mitogenic | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| LcH-A | | 60,000 | 2 | | | | | Yes (LcH-A) | |
| LcH-B | | 42,000 | 2 | | | | | | |
| ***Lepidium sativum*** (garden cress) | Cruciferae | 120,000 | 4 | 30,000 | 2.2% | None | NeuNAc, galNAc desialylated glycoproteins | | 254-255 |
| ***Lotononis bainesii*** | Leguminosae | | | | | | | | |
| Root | | 118,000 | | 32,000 35,000 | 12% (root) hexose (fuc, man, xylose, glcN) | | lac, lactulose, melibiose | | 256 |
| Seed | | 160-200,000 | | | 50% (seed) hexose | | gal, galactono-1,4-lactone | | |
| ***Lycopersicon esculentum*** (tomato) LEA | Solanaceae | 71,000 | | | 50% (arabinose 85%, gal 15%) | None | glcNAc oligomers | No | 257-260 |
| ***Maackia amurensis (Cladrastis amurensis)*** | Leguminosae | 130,000 | | | | | | | |
| MAH (strongly hemagglutinating) | | | 4 | 33,000 | 9.5% | | lac, NeuNAc | | 261 |
| MAM (strongly mitogenic) | | | 2 | 75,000 | 8.7% | | weak inhibitory activity | Yes | |

| Species | Family | Molecular weight (daltons) | Sub-units | Subunit size (daltons) | Carbohydrates | Specificity Human blood group | Specificity Sugar | Mitogenic | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| *Maclura pomifera* (Osage orange) MPA | Moraceae | 42,000 | | 10,000<br>12,000 | 0 | None | α-D-gal<br>galNAc | Yes | 262-267 |
| *Momordica charantia* (bitter pear melon) | Cucurbitaceae | 49,000<br>120,000<br>129,000 | 2 | 26,000<br>28,000<br>30,000 | 2% glc, 8% man, 2.5% glcN | O | D-gal, methyl-α-gal | | 268-272 |
| *Momordica dioica* | Cucurbitaceae | | | 23,000<br>37,000 | Yes | None | D-gal | | 273 |
| *Onobrychis viciifolia* (sainfoin) | Leguminosae | 53,000 | 2 | 26,000 | 2.6% n.s. | cat, rabbit | D-man,<br>D-glc<br>α-glycosidic derivatives | | 274-276 |
| *Ononis hircina* (restharrow) | Leguminosae | 110,000 | 4 | 31,000 | 7.2% n.s. | O | galNAc,<br>D-gal | No | 277 |
| *Oryza sativa* (rice) RBM, RGL | Gramineae | 44,000<br>19,000<br><br>23,000 | <br>2<br><br>2 | <br>8,200<br>11,000<br>11,300<br>13,700 | 0.8% | None | glcNAc | Yes | 278-280 |
| *Persea americana* (avocado) PAA | Lauraceae | | | | | A>B=O | basic proteins<br>basic polyamino acids | No | 281 |

| Species | Family | Molecular weight (daltons) | Sub-units | Subunit size (daltons) | Carbohydrates | Specificity Human blood group | Specificity Sugar | Mitogenic | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| ***Phaseolus limensis*** ***(P. lunatus, Lunatus limensis)*** (lima bean) LBA | Leguminosae | 247,100 124,400 | | 31,000 | 4% | A | α-D-galNAc | Yes | 282-288 |
| ***Phaseolus vulgaris*** (red kidney beans) PHA 5 isolectins $L_4, L_3E_1, L_1E_2, L_1E_3, E_4$ | Leguminosae | 115-140,000 | 4 | | | None | galNAc | | 289-304 |
| PHA-E | | 143,000 | 4 | 37,000 | | | | Low | |
| PHA-L | | 114,000 | 4 | 30,000 | 7.3% man, 2.8%glcN | | | High | |
| ***Phlomis fruticosa*** (Jerusalem sage) | Labiatae | | | | | A,B,AB | galNAc, D-gal | | 305 |
| ***Phytolacca americana*** (pokeweed, pigeon berry) PWA, PWM 5 isolectins, Pa-I to Pa-5 | Phytolaccaceae | 20-30,000 | | | 1.8-12.5% | None | glcNAc oligomers | | 306-312 |
| ***Pisum sativum*** (garden pea) PSA, PEA 2 isolectins | Leguminosae | 53,000 | | | | None | D-man, D-glc, glcNAc | Yes | 313-319 |
| I | | 54,000 | | 7,000 | 1.42% n.s. | | | | |
| II | | 53,000 | | 17,000 | 0.88% n.s. | | | | |

| Species | Family | Molecular weight (daltons) | Sub-units | Subunit size (daltons) | Carbohydrates | Specificity Human blood group | Specificity Sugar | Mitogenic | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| ***Psophocarpus tetragonolobus*** (winged bean) | Leguminosae | | | | | | | | |
| acidic I,II,III | | 54,000 | 2 | 27,000 | 7% (man, fuc, xylose, glcNAc) | | galNAc, D-gal | | 320-323 |
| basic B1,B2,B3 | | 58,000 | 2 | 29,000 | 5% (man, fuc, xylose, glcNAc) | A,B (trypsinized) | | | |
| ***Ricinus communis*** (castor bean) RCA | Euphorbiaceae | | | | | None | D-gal, galNAc | | 324-330 |
| RCA I | | 118- 120,000 | 4 | | | | | | |
| RCA II (toxin) | | 60- 65,000 | 2 | | | | | | |
| ***Robinia pseudoacacia*** (black locust, black acacia) RPA | Leguminosae | 100-110,000 | | 30,000 32,000 | 14-17% | None | galNAc | | 331-336 |
| ***Salvia horminum*** (annual clary, common sage) | Labiatae | | | | | Tn, Cad | | | 337-339 |
| ***Salvia sclarea*** (clary) SSA | Labiatae | | | | | Tn | | | 340-344 |

| Species | Family | Molecular weight (daltons) | Sub-units | Subunit size (daltons) | Carbohydrates | Specificity | | Mitogenic | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Human blood group | Sugar | | |
| *Sambucus nigra* (European elder) SNA | Caprifoliaceae | 140,000 | 2<br>2 | 34,500<br>37,500 | Yes | A>B>O | lac, galNAc | | 345 |
| *Secale cereale* (rye germ) | Gramineae | 36,000 | 2 | 18,000 | | None | glcNAc | | 346 |
| *Solanum tuberosum* (potato) STA | Solanaceae | 100,000 | 2 | 54,000 | 50% (92% arabinose) | None | glcNAc oligomers | | 347-354 |
| *Sophora japonica* (Japanese pagoda tree) | Leguminosae | 132,800 | 4 | 33,000 | 5.9% man, xylose | B>A | D-gal, galNAc | No | 355-361 |
| *Sorghum bicolor* | Gramineae | | | | | None | glcNAc, D-maltose | | 362 |
| *Tetragonolobus purpureas* *(Lotus tetragonolobus)* (winged or asparagus pea) 3 isolectins Lotus A | Leguminosae | 120,000<br>58,000<br>117,000 | | 27,000 | | O(H) | α-L-fuc | | 363-369 |
| *Triticum vulgaris* *(T. aestivum)* (wheat germ) several isolectins WGA | Gramineae | 36,000 | 2 | 18,000 | 0 | None | glcNAc oligomers | No | 370-379 |

| Species | Family | Molecular weight (daltons) | Sub-units | Subunit size (daltons) | Carbohydrates | Specificity: Human blood group | Specificity: Sugar | Mitogenic | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| ***Ulex europeus*** (gorse, furze, prickly broom) | Leguminosae | | | | | | | | 380-386 |
| UEA | | | | | | | | | |
| UEA I | | 170,000 | | | 5.2% (1.6% man, 1.4% glcN) | O | α-L-fuc | | |
| UEA II | | 23,000 | | | 21.7% (8.7% man, 6.3% gal) | O | N,N'-diacetylchitobiose | | |
| ***Vicia cracca*** (tufted or cow vetch) | Leguminosae | 125,000 | 4 | 33,000 | 5% | A | α-galNAc | | 387-393 |
| | | 44,000 | 2 | 5,700 | | None | man, glc | | |
| | | | 2 | 17,500 | | | | | |
| ***Vicia ervilia*** (bitter vetch) | Leguminosae | 60,000 | 2 | 21,000 | 0 | None | D-man, trehalose | | 394 |
| | | | 2 | 4,700 | | | | | |
| ***Vicia faba*** (fava or broad bean) | Leguminosae | 50,000 | 2 | 5,600 | 3% n.s. | None | D-man, D-glc | Yes | 395-400 |
| | | | 2 | 20,000 | | | | | |
| VFA | | 47,500 | 2 | 22,500 | | | | | |
| ***Vicia graminea*** | Leguminosae | 105,000 | 4 | 25,000 | 7.3% man, glcNAc | N | galNAc | No | 401-404 |
| VGA | | | | | | | | | |
| ***Vicia sativa*** (tare, spring vetch) | Leguminosae | 70,000 | 2 | 20,000 | 9.4% | None | melezitose | Yes | 405-407 |
| | | | | 27,500 | | | | | |
| VSL | | 40,000 | 2 | 6,000 | | | | | |
| | | | 2 | 14,000 | | | | | |

| Species | Family | Molecular weight (daltons) | Sub-units | Subunit size (daltons) | Carbohydrates | Specificity: Human blood group | Specificity: Sugar | Mitogenic | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| ***Vicia unijuga*** | Leguminosae | | | | | N | | | 408 |
| ***Vicia villosa*** | Leguminosae | 120,000 | 4 | 33,600 | 4.3% n.s. | | galNAc | | 409-411 |
| ***(Visea villosa)*** | | | | 35,900 | | | | | |
| (hairy or winter vetch) | | | | | 6.7-9.8% ($B_4$,$A_4$) | ($B_4$) Tn | | | |
| 3 isolectins | | | | | | ($A_4$) $A_1$ | | | |
| $B_4$,$A_2B_1$,$A_4$ | | | | | | | | | |
| VVA | | | | | | ($A_2B_1$) Intermediate between $A_4$,$B_4$ | | | |
| ***Vigna radiata*** | Leguminosae | 160,000 | 4 | 45,000 | | rabbit | α-D-galactosyl residues | | 412 |
| (mung bean) | | | | | | | | | |
| MBA | | | | | | | | | |
| ***Viscum album*** | Loranthaceae | | | | 10.1-11% | None | | | 413-419 |
| (European mistletoe) | | | | | | | | | |
| VAA | | | | | | | | | |
| I | | 115,000 | 2 | 29,000 | | | D-gal | | |
| | | | 2 | 34,000 | | | | | |
| II | | 60,000 | | 27,000 | | | D-gal, | | |
| | | | | 32,000 | | | galNAc | | |
| III | | 50,000 | | 25,000 | | | galNAc | | |
| | | | | 30,000 | | | | | |
| Wistaria floribunda | Leguminosae | 68,000 | 2 | 32,000 | 11.4% (5.8% man, 3.5% glcN) | None | galNAc | Yes | 420-425 |
| (Japanese wistaria) | | | | | | | | | |
| WFA | | | | | | | | | |

5) Biological Properties

Lectins display a variety of biological functions which enhance their usefulness. Properties such as the agglutination of erythrocytes and specific binding to carbohydrates are important features of a lectin.

The hemagglutinating activity of plant extracts that aroused early interest in lectins is well documented[18,426-436]. In some cases (*Dolichos biflorus, Arachis hypogaea, Griffonia simplicifolia* among others) this activity is blood group specific. In others, the agglutinin is specific for red blood cells of a particular species such as sheep (*Croton tiglium*), mouse or rat (*Lactuca scariole*) and cat or rabbit (*Onobrychis viciifolia*). Most lectins will agglutinate untreated erythrocytes but some require pretreatment of the red blood cells with neuraminidase (*Arachis hypogaea*), papain (*Cicer arietinum*) or trypsin (*Psophocarpus tetragonolobus*) for activity.

Lectins are also known for their sugar recognition specificities. They interact with the carbohydrate moieties of glycoproteins or glycolipids on cell surfaces[18,437] and induce cell-to-cell binding[438]. The ability to function as a lectin receptor is dependent upon the number and nature of sugar residues in the molecule[439]. Lectin binding may be affected by several factors including the presence of terminal sugar capping which may hinder binding[439], capacity of the adsorbent, temperature dependence, pH effects, ionic strength, concentration requirements for glycoside[440] and divalent metal ions[441].

This specific binding, as described above, produces a number of biological effects. Two of the most studied are the mitogenic stimulation of lymphocytes[8,9,15,16,18,446-449] and the agglutination of transformed cells[7-9,15,16,451-457]. Several other unique properties reported include:

- Insulin-like activity[442] and the stimulation of insulin release[16,443]
- Inhibition of interferon action[444], fungal growth[18,445] and epinephrine-stimulated lipolysis[270]

- Suppression of antibody response[450]
- Binding to sperm[458] and interactions with developing cells[9,15]
- Induction of the platelet release reaction[16,459]
- Inhibition of phagocytosis and protein synthesis[459,460].

6) Applications

In view of their unique biological properties, a number of clinical and research-oriented applications for lectins have emerged:

a) Clinical

- Routine and theoretical investigations of blood grouping and detection of "secretors"
- Chromosome analysis and fractionation of lymphocyte sub-populations
- Histopathological studies and comparative investigations on normal and transformed cells
- Immunohistochemical probes for metabolic and glycoprotein storage diseases
- Target-oriented therapy of malignant tumors
- Detection and identification of human pathogens

b) Research

- Isolation and purification of glycoproteins, glycopeptides and glycosylated nucleic acids by affinity chromatography
- Cell separations
- Identification and quantification of glycoproteins
- Structural studies on carbohydrates and glycoproteins using lectins and lectin-resistant cell surface variants of eukaryotic cells and selected cell lines with altered membrane carbohydrates

- Probes for the characterization of cell surfaces and the examination of membrane topography
- Eliciting monoclonal antibodies
- Retrograde and anterograde study of neural transport
- Plant chemotaxonomy

Lectins have been widely utilized as blood typing reagents in immunohematology[461]. Plant agglutinins have been used to determine the antigenic relations of blood group antigens[421] and the diagnosis of blood subgroups A and AB[462]. Secretors and non-secretors can be distinguished by *Ulex europeus* agglutinin[462].

The preparation of metaphase chromosomes from lymphoid cells is facilitated by mitogenic stimulation with lectins such as *Phaseolus vulgaris* agglutinin. Structural analysis of stained chromosome bands allows the detection of environmentally-induced and inherited genetic abnormalities, which manifest themselves in pathological conditions such as Klinefelter's, Turner's, Down's, Patau's and Edwards' syndromes. Lymphocytes have been fractionated using lectin binding techniques that capitalize on the surface differences between normal lymphocytes and those modified by lymphoproliferative diseases[463]. Selective agglutination is also the basis for separation of mouse lymphocyte classes[464-466].

Lectins are useful histochemical probes with potential as diagnostic tools. Tumors and metastases can be detected using radioiodinated lectins and monoclonal antibodies. Agglutinins conjugated to fluorochromes, peroxidase, ferritin or biotin, can localize glycosidic components of cells and trace their changes in the pathological state[467] of a variety of tissues[468]. While the emphasis has been on malignant cells[469,470], lectins have also been employed in other studies, such as the differentiation of normal and psoriatic epidermis[471].

The diagnostic use of lectins has been extended to the identification of glycoprotein storage diseases[472,473]. Lectin reagents are believed to be cheaper and simpler than conventional biochemical tests needed to identify specific sugars in undegraded stored

substances[472] or demonstrate a deficiency in enzyme activity.

Target-oriented therapy of malignant tumors using enzymes or drugs coupled to lectins may provide a new approach to chemotherapy[56]. Lectin derivatives of methotrexate and chlorambucil showed greater inhibitory effects on tumor cell DNA biosynthesis than equivalent doses of free drug and lectin[474]. Fluorescent-labelled lectin derivatives may also have value in the identification and detection of human fungal pathogens[475].

Agglutinins have many research applications in addition to their clinical potential. Immobilized lectins linked to inert supports have been used in the isolation and purification of glycoproteins, glycopeptides and glycosylated nucleic acids by affinity chromatography[8,16,18,439,440,476].

Affinity chromatography can also be used for cell separation, along with selective agglutination, mixed rosetting and flow microfluorimetry techniques. This application of lectins has been limited to the separation of mammalian cells, especially lymphocytes, but offers the advantage of high yields of viable cells since the binding of lectins to cells is reversible without damage to the cells[477].

Enzyme-linked lectin assays (ELLA), similar to the enzyme-linked immunosorbent assay (ELISA) but based on nonimmunologic reagents, have been developed as specific probes for end groups on immobilized glycoproteins[478,479]. Quantification can be achieved using rocket affinoelectrophoresis in a gel containing lectin[480,481]. The amount of glycoprotein applied and the rocket height correlate[481].

Structural studies on carbohydrates have been conducted using various combinations of immobilized lectin columns to fractionate sugar chains into various structural groups. Absolute configurations of sugars have been determined on the basis of inhibition of $^{125}$I-labelled lectin binding[482]. Structural assessments of glycosylated macromolecules have also been achieved using lectin-resistant cell surface variants of eukaryotic cells and selected cell

lines with altered membrane carbohydrates.

Lectin probes have been employed in the characterization of cell surfaces and the examination of membrane topography[484]. The diversity of investigations ranges from cell surface glycoconjugates and membrane anomalies[485-488] to probing membrane asymmetry and compartmentalization[489] and the binding of glycoproteins from microsomal and Golgi membranes to lectins[490].

Agglutinins can be used to induce "capping", an effect characterized by the displacement of antigenic molecules to one pole of the cell. This phenomenon allows membrane fluidity to be studied.

Other research applications of lectins include the elicitation of monoclonal antibodies[491], retrograde and anterograde study of neural transport[492] and the chemotaxonomic classification of plants based on N-terminal sequence data[148].

**B. Microorganisms and Lectins**

A number of bacterial species have been shown to interact with lectins[493]. The binding of lectins to microbial cell walls involves a complex reaction[494]. Concanavalin A is known to agglutinate bacterial spheroplasts[495] and interact specifically with bacterial cell walls containing the appropriate glycosidic components on their teichoic acids[496]. Concanavalin A and wheat germ agglutinin both agglutinate *Streptococci*[497-501]. Lectins have been used to study the surface carbohydrates of various *Mycoplasma* membranes as well[502-505]. Lectins will disrupt the attachment and internalization of *Chlamydia*[506-508]. Other studies have demonstrated the interaction of lectins with *Salmonella*[509-510], *Staphylococcus*[511], *Mycobacterium*[512], *Bacillus*[513], *Legionella*[514], *Haemophilus*[515], *Escherichia*[516], *Campylobacter*[517] and *Pseudomonas*[518].

While lectins can agglutinate and interact with bacteria, the bacteria themselves also have lectins[519-525]. Fimbriae (pili) and some outer membrane proteins are known to contain bacterial surface lectins[526-528]. These lectins have been postulated to mediate the adherence of bacteria to epithelial cells[529-531] and may be involved in the initial stages of bacterial infection [532-535]. Bacterial lectins also bind lymphocytes[536-537] and may act as determinants of recognition in phagocytosis[538]. Bacterial-induced histamine release in intrinsic asthma has been attributed to bacterial lectins[539-540].

Lectin-microbe interactions are potentially useful for distinguishing bacterial species and identifying particular sugar residues on bacterial cell surfaces. In particular, the interaction of lectins with *Neisseria gonorrhoeae*[541-543] has lead to the epidemiological characterization of this bacterium[544] and rapid diagnostic tests for laboratory identification[545-547]. The differentiation of *Bacillus* species[548-549], *Brucella* strains[550], coagulase positive and negative *Staphylococci*[551] and detection of group C *Streptococci*[552] have also been achieved using lectins. Lectin-carbohydrate interactions have been used to quantify microbial cells[553] and investigate receptors for bacterial toxins[554].

Although limited studies have been reported on bacterial lectins and the use of lectins in microbiology, even less is known about agglutinins and viruses. A sialic acid binding hemagglutinin has been identified in influenza virus. Given the potential for antigenic shift and drift in this virus, lectins gain importance in clinical epidemiology particularly since the lectin part may change.

Lectins are reported to agglutinate cells following infection with nononcogenic viruses[556-560], and transformed avian RNA tumor viruses[561-562]. This property of lectins to induce viral agglutination has been used to purify oncornaviruses[563] and viral glycoproteins[555].

Lectins are also known to affect the growth and release of viruses[564,565], including myxovirus. The cytopathogenicity of Newcastle disease virus was modified by agglutinins[566,567]. Similar treatment with Con A rendered Sendai virus and herpes simplex virus non-infectious[568].

## C. Botany of the Moraceae Family

The Urticales (nettle) order is comprised of approximately 125 genera and 3,100 species in the Ulmaceae (elm), Moraceae (mulberry), Cannabinaceae (hemp) and Urticaceae (nettle) families[569]. The Moraceae family, consisting of about 75 genera and 1850 species, is distributed mainly in tropical and subtropical regions[570]. Corner[571] describes six tribes (Ficeae, Dorstenieae, Brosimeae, Moreae, Olmedieae and Artocarpeae) whereas Hutchinson[570] lists three subfamilies and nine tribes (Moroideae: Fatoueae, Moreae, Broussonetieae, Strebleae, Dorstenieae; Artocarpoideae: Euartocarpeae, Olmedieae, Brosimeae, Ficeae; and Conocephaloideae). *Ficus* represents the largest genus with more than 800 species. *Dorstenia* contains about 170 species while *Brosimum* and *Artocarpus* each number around 50 species. Another 12 Moraceae genera contain ten to 25 species each, while the remaining genera have less than four species each.

Moraceae plants generally contain a milky latex. The leaves tend to be simple and alternate and the flowers small and apetalous. Many species possess multiple fruits that result from different flowers fusing together to form an aggregate fruit[569,572-574]. Economically the family is important for edible fruit (*Artocarpus*, *Ficus*, *Treculia*, *Morus*, *Chlorophora*), timber (*Ficus*, *Antiaris*, *Castilloa*, *Musanga*, *Brosimum*), latex products such as components in rubber, glue and caulking compounds and an arrow poison (*Ficus*, *Artocarpus*, *Castilloa*, *Cecropia*, *Antiaris*), ornamentals (*Maclura*, *Ficus*, *Dorstenia*, *Cudrania*, *Chlorophora*), fibres for bark cloth or paper (*Artocarpus*, *Broussonetia*) and leaves for feeding silkworms (*Morus*, *Maclura*[570,572]).

*Artocarpus* plants are monoecious, with both male and female flowers on the same plant, and contain a sticky, white latex[573]. The leaves alternate or form a spiral arrangement. The fruit is a fleshy, pulpy syncarp from eight to about 20 cm in diameter, containing many seeds which lack an endosperm[573,575]. The genus has been thoroughly described by Fosberg[576] and Jarrett[577-579]. Trees and shrubs of the genus *Ficus* are also monoecious flowers, have alternate leaves and produce latex[573,575].

**D. Aims and Objectives of Current Investigation**

Most previous investigations of lectin activity have focused primarily on lectins of the Leguminosae family, because of the economic importance of this taxa and abundance of agglutinating material in seeds of these plants. In the case of the Moraceae family, the lectins have not been studied or characterized extensively with the exception of *Maclura pomifera* (Osage orange) and *Artocarpus integrifolia* (jackfruit). One of the objectives of this study was to obtain more information on agglutinating substances in this botanical group.

Initial studies were directed towards the screening of lectins for serological cross-reactivity and relatedness to known lectins and the evaluation of crude extracts from new plant sources for potentially useful lectins. Based on this screening, the objective was to identify, purify and characterize new Moraceae lectins. The intent was to find new lectins which could have applications in the field of microbiology. Gram negative bacilli such as *Escherichia*, *Proteus* and *Pseudomonas* species, are part of the normal endogenous flora. However, these organisms can cause opportunistic infections upon leaving their normal habitat or after their introduction into debilitated patients. *Proteus* strains show a high degree of multiple drug resistance and *Pseudomonas* species are known to resist most antimicrobial agents. The cell wall of a bacterium exhibits many dynamic functions including rigidity and shape determination, protection and provision of a site of antigenic specificity. Its role as a permeability barrier may play a significant part in resistance to antibiotics. The identification and characterization of new lectins will increase the number of useful probes for studying the binding sites and moieties on the cell walls of drug resistant bacteria. This information should contribute to the design of better, more effective antimicrobial agents and the development of diagnostic kits.

A secondary objective involved the evaluation of different techniques and the application of new developments in protein chemistry to the purification and study of plant lectins.

**E. References**


1. STILLMARK, H. *Arch. Pharmakol. Inst. Dorpat* **3**: 59-72, 1889.

2. SHARON, N. and LIS, H. In: Methods in Membrane Biology .Vol. 3, Plasma Membranes. Edited by E.D. Korn. New York: Plenum Press, 1975. pp. 147-200.

3. BOYD, W.C. *Vox Sang.* **8**: 1-32, 1963.

4. DECHARY, J.M. *Vox Sang.* **15**: 401-409, 1968.

5. BOYD, W.C. *Ann. N.Y. Acad. Sci.* **169**: 168-190, 1970.

6. TOM, G.C. and WESTERN, A. In: Chemotaxonomy of the Leguminosae. Edited by J.B. Harborne, D. Boulter and B.L. Turner. London: Academic Press, 1971. pp. 367-462.

7. SHARON, N. and LIS, H. *Science* **177**: 949-959, 1972.

8. LIS, H. and SHARON, N. *Ann. Rev. Biochem.* **42**: 541-574, 1973.

9. NICOLSON, G.L. *Int. Rev. Cytol.* **39**: 89-190, 1974.

10. HUGHES, R.C. In: Membrane Glycoproteins - A, Review of Structure and Function. London: Butterworth & Co. Ltd., 1976. pp. 135-151.

11. LIENER, I.E. *Ann. Rev. Plant Physiol.* **27**: 291-319, 1976.

12. SHARON, N. *Sci. Amer.* **236**(6): 108-119, 1977.

13. LIS, H. and SHARON, N. In: The Antigens. Vol. IV. Edited by M. Sela. New York: Academic Press, 1977. pp. 429-529.

14. DULANEY, J.T. *Mol. Cell. Biochem.* **21**(1): 43-63, 1978.

15. BROWN, J.C. and HUNT, R.C. *Int. Rev. Cytol.* **52**: 277-349, 1978.

16. ROTH, J. *Experimentelle Pathologie, Suppl.* **3**: 7-186, 1978.

17. GOLDSTEIN, I.J. and HAYES, C.E. *Adv. Carbohydr. Chem. Biochem.* **35**: 127-340, 1978.

18. LIS, H. and SHARON, N. In: The Biochemistry of Plants . Vol. 6, Proteins and Nucleic Acids. Edited by A. Marcus. New York: Academic Press, 1981. pp.371-447.

19. ETZLER, M.E. *Prog. Clin. Biol. Res.* **138**: 1-5, 1983.

20. BOYD, W.C. In: The Proteins - Chemistry, Biological Activity and Methods. Vol. II, Pt. B. New York: Academic Press, 1954. pp. 756-844.

21. GOLDSTEIN, I.J., HUGHES, R.C., MONSIGNY, M., OSAWA, T. and SHARON, N. *Nature* **285**: 66, 1980.

22. KOCOUREK, J. and HOREJSI, V. *Nature* **290**: 188, 1981.

23. KOCOUREK, J. and HOREJSI, V. In: Lectins - Biology, Biochemistry, Clinical Biochemistry. Vol. 3. Edited by T.C. Bog-Hansen and G.A. Spengler. Berlin: W. de Gruyter & Co., 1983. pp. 3-6.

24. DIXON, H.B.F. *Nature* **292**: 192, 1981.

25. NC-IUB. Newsletter. *J. Biol. Chem.* **256**(1): 12-14, 1981.

26. PROKOP, 0., UHLENBRUCK, G., ROTHE, A. and COHEN, E. Ann. *N.Y. Acad. Sci.* **234**: 228-231, 1974.

27. LEVI, G. and TEICHBERG, V.I. *FEBS Letters* **148**(1):145-148, 1982.

28. TEICHBERG, V.I. *Methods Enzymol.* **50**: 291-302, 1978.

29. RICE, R.H. and ETZLER, M.E. *Biochem.* **14**(18): 4093-4099, 1975.

30. WRIGHT, C.S., GAVILANES, F. and PETERSON, D.L. *Biochem.* **23**: 280-287, 1984.

31. LAMB, J.E. and GOLDSTEIN, I.J. *Arch. Biochem. Biophys.* **229**(1): 15-26, 1984.

32. LEAVITT, R.D., FELSTED, R.L. and BACHUR, N.R. *J. Biol. Chem.* **252**(9): 2961-2966, 1977.

33. VRETBLAD, P., HJORTH, R. and LAAS, T. *Biochem. Biophys. Acta* **579**: 52-61, 1979.

34. KILPATRICK, D.C., WESTON, J. and URBANIAK, S.J. *Anal. Biochem.* **134**: 205-209, 1983.

35. FITZGERALD, J.E., CATT, J.W. and HARRISON, F.L. *Eur. J. Biochem.* **140**(1): 137-141, 1984.

36. ALLEN, A.K., NEUBERGER, A. and SHARON, N. *Biochem. J.* **131**: 155-162, 1973.

37. PUSZTAI, A. and WATT, W.B. *Biochim. Biophys. Acta* **365**(1): 57-71, 1974.

38. FOUNTAIN, D.W. and YANG, W.K. *Biochim. Biophys. Acta* **492**(1): 176-185, 1977.

39. MURPHY, L.A. and GOLDSTEIN, I.J. *J. Biol. Chem.* **252**(13): 4739-4742, 1977.

40. FELSTED, R.L., LEAVITT, R.D., CHEN, C., BACHUR, N.R. and DALE, R.M.K. *Biochim. Biophys. Acta* **668**(1): 132-140, 1981.

41. COOPER, D.N. and BARONDES, S.H. *J. Biol. Chem.* **256**(10): 5046-5051, 1981.

42. PUEPPKE, S.G. *Arch. Biochem. Biophys.* **212**(1): 254-261, 1981.

43. FRANZ, H. and ZISKA, P. In: Lectins - Biology, Biochemistry, Clinical Biochemistry. Vol. 1. Edited by T.C. Bog-Hansen. Berlin: W. de Gruyter & Co., 1981. pp. 17-21.

44. FRANZ, H., BERGMANN, P. and ZISKA, P. *Histochem.* **59**: 335-342, 1979.

45. FRANZ, H., ZISKA, P. and MOHR, J. *Acta Histochem.* **71**: 19-21, 1982.

46. PAPPENHEIMER, A.M. Jr., OLSNES, S. and HARPER, A.A. *J. Immunol.* **113**(3): 835-841, 1974.

47. UHLENBRUCK, G. *Naturwissenschaften* **68**: 606-612, 1981.

48. FRANZ, H. . *Acta Histochem., Suppl.* **22**: 123-125, 1980.

49. HAKOMORI, S.-I. In: Monoclonal Antibodies and Functional Cell Lines Progress and Applications. Edited by R.H. Kennett, K.B. Bechtol and T.J. McKearn. New York: Plenum Press, 1984. p. 69.

50. GALLAGHER, J.T. *Biosci. Rep.* **4**: 621-632, 1984.

51. BALDING, P. In: Lectins - Biology, Biochemistry, Clinical Biochemistry. Vol. 1. Edited by T.C. Bog-Hansen. Berlin: W. de Gruyter & Co., 1981. pp. 11-16.

52. MEADE, N.A., STAAT, R.H., LANGLEY, S.D. and DOYLE, R.J. *Carbohydr. Res.* **78**(2): 349-363, 1980.

53. BOYD, W, C. and REGUERA, R.M. *J. Immunol.* **62**: 333-339, 1949.

54. BOYD, W.C., WASZCZENKO-ZACHARCZENKO, E. and GOLDWASSER, S.M. *Transfusion* **1**: 374-382, 1961.

55. ALLEN, N.K. and BRILLIANTINE, L. *J. Immunol.* **102**(5): 1295-1299, 1969.

56. SANDHU, R.S. and REEN, R.S. In: Lectins - Biology, Biochemistry, Clinical Biochemistry. Vol.2. Edited by T.C. Bog-Hansen. Berlin: W. de Gruyter & Co., 1982. pp.113-136.

57. SANDHU, R.S., REEN, R.S., SINGH, S., SINGH, J. and CHOPRA, S.K. In: Lectins Biology, Biochemistry, Clinical Biochemistry. Vol.2. Edited by T.C. Bog-Hansen. Berlin: W. de Gruyter & Co., 1982. pp. 679-692.

58. NACHBAR, M.S. and OPPENHEIM, J.D. *Amer. J. Clin. Nutr.* **33**: 2338-2345, 1980.

59. GOLD, E.R. and BALDING, P. Receptor-Specific Proteins - Plant and Animal Lectin. Amsterdam: Excerpta Medica, 1975.

60. SIMPSON, D.L., THORNE, D.R. and LOH, H.H. *Life Sciences* **22**: 727-748, 1978.

61. ROBERTS, T.K. and BOURSNELL, J.C. *J. Reprod. Fert.* **41**: 489-492, 1974.

62. STOCKERT, R.J., MORELL, A.G. and SCHEINBERG, I.H. *Science* **186**: 365-366, 1974.

63. COHEN, E. (editor). *Prog. Clin. Biol. Res.* **157**, 1984.

64. BIRD, G.W.G. *Ann. N.Y. Acad. Sci.* **234**: 51-54, 1974.

65. INGRAM, G.A. *Dev. Comp. Immunol.* **9**(1): 1-10, 1985.

66. INGRAM, G.A. *Dev. Comp. Immunol.* **9**(3): 385-394, 1985.

67. ROSEN, S.D., REITHERMAN, R.W. and BARONDES, S.H. *Exp. Cell Res.* **95**(1): 159-166, 1975.

68. BARTLES, J.R., FRAZIER, W.A. and ROSEN, S.D. *Int. Rev. Cytol.* **75**: 61-99, 1982.

69. GILBOA-GARBER, N. *Biochim. Biophys. Acta* **273**: 165-173, 1972.

70. FUJITA, Y., OISHI, K. and AIDA, K. *Biochem. Biophys. Res. Comm.* **53**: 495-501, 1973.

71. FUJITA, Y., OISHI, K. and AIDA, K. *J. Biochem.* **76**: 1347-1349, 1974.

72. HOWE, M.L. and BARRETT, J.T. *Biochim. Biophys. Acta* **215**: 97-104, 1970.

73. COOFER, E.L., LEMMI, C.A.E. and MOORE, T.C. *Ann. N.Y. Acad. Sci.* **234**: 34-50, 174.

74. KOTHBAUER, H. and SCHENKEL-BRUNNER, H. *Comp. Biochem. Physicol.* **A50**: 27-29, 1975.

75. PAULEY, G.B. *Ann. N.Y. Acad. Sci.* **234**: 145-160, 1974.

76. PEMBERTON, R.T. *Ann. N.Y. Acad. Sci.* **234**: 95-121, 1974.

77. LIENER, I.E. *J. Agr. Food Chem.* **22**(1): 17-22, 1974

78. TURNER, R.H. and LIENER, I.E. *J. Agric. Food Chem.* **23**(3): 484-487, 1975.

79. BARONDES, S.H. *Science* **223**: 1259-1264, 1984.

80. ETZLER, M.E. *Prog. Clin. Biol. Res.* **138**: 197-207, 1983.

81. ROBERTS, D.M. and ETZLER, M.E. *Plant Physiol.* **76**: 879-884, 1984.

82. HOWARD, I.K., SAGE, H.J. and HORTON, C.B. *Arch. Biochem. Biophys.* **149**: 323-326, 1972.

83. STINISSEN, H.M., CHRISFEELS, M.J. and PEUMANS, W.J. *Planta* **164**: 278-286, 1985.

84. KHANNA, A.K. and SEHAJPAL, P.K. *Vox Sang.* **39**: 44-45, 1980.

85. HOROWITZ, J. *Mol. Gen. Genet.* **198**: 482-485, 1985.

86. STINISSEN, H.M., PEUMANS, W.J., LAW, C.N. and PAYNE, P.I. *Theor. Appl. Genet.* **67**: 53-58, 1983.

87. RUDIGER, H. In: Lectins - Biology, Biochemistry, Clinical Biochemistry. Vol. 1. Edited by T.C. Bog-Hansen. Berlin: W. de Gruyter & Co., 1981. pp. 3-10.

88. MARX, J.L. *Science* **196**: 1429-1478, 1977.

89. KAUSS, H. and GLASER, C. *FEBS Letters* **45**(1): 304-307, 1974.

90. HAMBLIN, J. and KENT, S.F. *Nature New Biology* **245**: 28-30, 1973.

91. BAUER, W.D. *Ann. Rev. Plant Physiol.* **32**: 407-449, 1981.

92. DAZZO, F.B. and TRUCHET, G.L. *J. Membr. Biol.* **73**: 1-16, 1983.

93. DAZZO, F.B. and HUBBELL, D.H. *Appl. Microbiol.* **30**: 1017-1033, 1975.

94. JANZEN, D.H., JUSTER, H.B. and LIENER, I.E. *Science* **192**(4241): 795-796, 1976.

95. ALBERSHEIM, P. and ANDERSON, A.J. *Proc. Nat. Acad. Sci. U.S.A.* **68**(8): 1815-1819, 1971.

96. BRAMBL, R. and GADE, W. *Physiologia Plantarum* **64**(3): 402-408, 1985.

97. TALBOT, C.F. and ETZLER, M.E. *Biochem.* **17**: 1474-1479, 1978.

98. SHANNON, L.M., HANKINS, C.N. and STROSBERG, A.D. In: Lectins - Biology, Biochemistry, Clinical Biochemistry. Vol. 1. Edited by T.C. Bog-Hansen. Berlin: W. de Gruyter & Co., 1981. pp. 81-91.

99. NEUBERGER, A. *Biol. Cell* **51**: 113-114, 1984.

100. BLAESE, R.M. FLEISHER, T.A. TOSATO, G. MUCHMORE, A.V. and GREENE, W.C. *Amer. J. Ped. Hematol. Oncol.* **5**(2): 199-206, 1983.

101. CLARKE, A.E. and KNOX, R.B. *Quart. Rev. Bio.* **53**: 3-28, 1978.

102. ANDERSON, M.A., HOGGART, R.D. and CLARKE, A.E. *Prog. Clin. Biol. Res.* **138**: 143-161, 1983.

103. BARONDES, S.H. *Ann. Rev. Biochem.* **50**: 207-231, 1981.

104. OCHOA, J.-L., SIERRA, A. and CORDOBA, F. In: Lectins - Biology, Biochemistry, Clinical Biochemistry. Vol. 1. Edited by T.C. Bog-Hansen. Berlin: W. de Gruyter & Co., 1981. pp. 73-80.

105. WEI, C.H., HARTMAN, F.C., PFUDERER, P. and YANG, W.-K. *J. Biol. Chem.* **249**: 3061-3067, 1974.

106. LIN, J.-Y., LEE, T.-C., HU, S.-T. and TUNG, T.-C. *Toxicon* **19**: 41, 1981.

107. OLSNES, S. and PIHL, A. *Eur. J. Biochem.* **35**: 179-185, 1973.

108. WEI, C.H., KOH, C., PFUDERER, P. and EINSTEIN, J.R. *J. Biol. Chem.* **250**: 4790-4795, 1975.

109. KAUFMAN, S.J. and McPHERSON, A. *Cell* **4**: 263-268, 1975.

110. OLSNES, S., SALTVEDT, E. and PIHL, A. *J. Biol. Chem.* **249**: 803-810, 1974.

111. ROY, J., SOM, S. and SEN, A. *Arch. Biochem. Biophys.* **174**(1): 359-361, 1976.

112. HERRMANN, M.S. and BEHNKE, W.D. *Biochim. Biophys. Acta* **621**(1): 43-52, 1980.

113. BARBIERI, L., ZAMBONI, M., MONTANARO, L., SPERTI, S. and STIRPE, F. *Biochem. J.* **185**: 203-210, 1980.

114. OLSNES, S., HAYLETT, T. and SANDVIG, K. *Methods Enzymol.* **83**: 357-362, 1982.

115. REFSNES, K., HAYLETT, T., SANDVIG, K. and OLSNES, S. *Biochem. Biophys. Res. Comm.* **79**: 1176-1183, 1977.

116. OLSNES, S., HAYLETT, T. and REFSNES, K. *J. Biol. Chem.* **253**(14): 5069-5073, 1978

117. OLSNES, S., SANDVIG, K., EIKLID, K. and PIHL, A. *J. Supramol. Struct.* **9**: 15,25, 1978.

118. GASPERI-CAMPANI, A., BARBIERI, L., LORENZONI, E., MONTANARO, L., SPERTI, S., BONETTI, E. and STIRPE, F. *Biochem. J.* **174**: 491-496, 1978.

119. PEUMANS, W.J., NSIMBA-LUBAKI, M., PEETERS, B. and BROEKAERT, W.F. *Planta* **164**(1): 75-82, 1985.

120. CAMMUE, B., STINISSEN, H.M. and PEUMANS, W.J. . *Eur. J. Biochem.* **148**(2): 315-322, 1985.

121. SUZUKI, I., SAITO, H., INOUE, S., and TAKAHASHI, T. *J. Biochem.* **85**(1): 163-171, 1979.

122. BLACIK, L.J., BREEN, M., WEINSTEIN, H. G. SITTIG, R.A. and COLE, M. *Biochim. Biophys. Acta* **532**(2): 225-231, 1978.

123. BIRD, G.W.G. *Vox Sang.* **9**: 748-749, 1964.

124. LOTAN, R., SKUTELSKY, E., DANON, D. and SHARON, N. *J. Biol. Chem.* **250**: 8518-8523, 1975.

125. LOTAN, R. and SHARON, N. *Methods Enzymol.* **50**: 361-367, 1978.

126. LONDON, J., BERRIH, S. and BACH, J.-F. *J. Immunol.* **121**(2): 438-443, 1978.

127. IRIMURA, T., KAWAGUCHI, T., TERAO, T. and OSAWA, T. *Carbohydr. Res.* **39**: 317-327, 1975.

128. NOVOGRODSKY, A., LOTAN, R., RAVID, A. and SHARON, N. *J. Immunol.* **115**(5): 1243-1248, 1975.

129. TERAO, T., IRIMURA, T. and OSAWA, T. *Hoppe Seylers Z, Physiol. Chem.* **356**: 1685-1692, 1975.

130. SPRINGER, G.D., DESAI, P.R. and BANATWALA, I. *Die Naturwissenschaften* **61**: 457-458, 1974.

131. DECHARY, J.M., LEONARD, G.L. and CORKERN, S. *Lloydia* **33**: 270-274, 1970.

132. PEREIRA, M.E., KABAT, E.A., LOTAN, R. and SHARON, N. *Carbohydr. Res.* **51**(1): 107-118, 1976.

133. LOTAN, R., LIS, H. and SHARON, N. *Biochem. Biophys. Res. Comm.* **62**(1): 144-150, 1975.

134. SOARES, M.B.M., ARMADA, J.L.A., SOARES, V.M.F.C. and SEUANEZ, H.N. *Cytobios* **34**: 165-174, 1982.

135. KUMAR, G.S., APPUKUTTAN, P.S. and BASU, D. *J. Biosci.* **4**(3): 257-261, 1982.

136. APPUKUTTAN, P.S. and BASU, D. *FEBS Letters* **180**(2): 331-334, 1985.

137. NAMJUNTRA, P., MUANWONGYATHI, P. and CHULAVATNATOL, M. *Biochem. Biophys. Res. Comm.* **128**(2): 833-839, 1985.

138. DE, AZEVEDO, MOREIRA, R. and AINOUZ, I.L. *Plant Physiol.* **61**(suppl. 118): 118, 1978.

139. APPUKUTTAN, P.S. and BASU, D. *J. Biosci.* **7**(1): 7-14, 1985.

140. BUNN-MORENO, M.M. and CAMPOS-NETO, A. *J. Immunol.* **127**(2): 427-429, 1981.

141. ROQUE-BARREIRA, M.C. and CAMPOS-NETO, A. *J. Immunol.* **134**(3): 1740-1743, 1985.

142. RESTUM-MIGUEL, N. and PROUVOST-DANON, A. *Immunol.* **54**: 497-504, 1985.

143. CHATTERJEE, B.P. and UHLENBRUCK, G. *Experientia* **38**: 1225-1226, 1982.

144. OSAWA, T., IRIMURA, T. and KAWAGUCHI, T. *Methods Enzymol.* **50**: 367-372, 1978.

145. IRIMURA, T. and OSAWA, T. *Arch. Biochem. Biophys.* **151**: 475-482, 1972.

146. BOYD, W.D., EVERHART, D.L. and McMASTER, M.H. *J. Immunol.* **81**: 414-418, 1958.

147. UHLENBRUCK, G. and DAHR, W. *Vox Sang.* **21**: 338-351, 1971.

148. YOUNG, N.M., WATSON, D.C. and WILLIAMS, R.E. *FEBS Letters* **182**(2): 403-406, 198

149. PEUMANS, W.J., SPAEPEN, C., STINISSEN, H.M. and CARLIER, A.R. *Biochem. J.* **205**(3): 635-638, 1982.

150. HOREJSI, V., TICHA, M., NOVOTNY, J. and KOCOUREK, J. *Biochim. Biophys. Acta* **623**: 439-448, 1980.

151. TUMOSA, C.S. *Experientia* **40**(7): 718-719, 1984.

152. TUMOSA, C.S. *Transfusion* **24**(5): 414, 1984.

153. MCKENZIE, G.H., SAWYER, W.H. and NICHOL, L.W. *Biochim. Biophys. Acta* **263**: 283-293, 1972.

154. MCCUBBIN, W.D. and KAY, C.M. *Biochem. Biophys. Res. Comm.* **44**(1): 101-109, 1971.

155. REEKE, G.N., BECKER, J.W., CUNNINGHAM, B.A., GUNTHER, G.R., WANG, J.L. and EDELMAN, G.M. *Ann. N.Y. Acad. Sci.* **234**: 369-382, 1974.

156. KALB, A.J. and LUSTIG, A. *Biochim. Biophys. Acta* **168**: 366-367, 1968.

157. GUNTHER, G.R., WANG, J.L., YAHARA, I., CUNNINGHAM, B.A. and EDELMAN, G.M. *Proc. Natl. Acad. Sci.* **70**: 1012-1016, 1973.

158. BITTIGES, H. and SCHNEBLI, H.P. (editors). Concanavalin A, as a tool. London: J. Wiley & Sons, 1976.

159. AGRAWAL, B.B.L. and GOLDSTEIN, I.J. *Arch. Biochem. Biophys.* **124**: 218-229 1968.

160. SUMNER, J.B. and HOWELL, S.F. *J. Bacteriol.* **32**: 227-237, 1936.

161. SUMNER, J.B., GRALEN, N. and ERIKSSON-QUENSEL, I.-B. *J. Biol. Chem.* **125**: 45-48, 1938.

162. AGRAWAL, B.B.L. and GOLDSTEIN, I.J. *Biochem. J.* **96**: 23-25, 1965.

163. AGRAWAL, B.B.L. and GOLDSTEIN, I.J. *Methods Enzymol.* **28**: 313-318, 1972.

164. PORETZ, R.D. and GOLDSTEIN, I.J. *Biochem.* **9**: 2890-2896, 1970.

165. YARIV, J., KALB, A. J. and LEVITZKI, A. *Biochem. Biophys. Acta* **165**: 303-305, 1968.

166. HARDMAN, K.D. and GOLDSTEIN, I.J. In: Immunochemistry of Proteins. Vol. 2. Edited by M.Z. Atassi. New York: Plenum Press, 1977. pp. 373-417.

167. BLOCH, R., JENKINS, J., ROTH, J. and BURGER, M.M. *J. Biol. Chem.* **251**(19): 5929-5935, 1976.

168. BIRD, G.W.G. and WINGHAM, J. *Vox Sang.* **19**: 132-139, 1970.

169. PEUMANS, W.J., DELEY, M., STINISSEN, H.M. and BROEKAERT, W.F. *Plant Physiol.* **78**(2): 379-383, 1985.

170. KOLBERG, J., MICHAELSEN, T.E. and SLETTEN, K. *Hoppe Seylers Z. Physiol. Chem.* **364**(6): 655-664, 1983.

171. KITAGAKI, H., SENO, N., YAMAGUCHI, H. and MATSUMOTO, I. *J. Biochem.* **97**(3): 791-799, 1985.

172. ERSSON, B., ASPBERG, K. and PORATH, J. *Biochim. Biophys. Acta* **310**: 446-452, 1973.

173. ERSSON, B. *Biochim. Biophys. Acta* **494**(1): 51-60, 1977.

174. BANERJEE, K.K. and SEN, A. *Arch. Biochem. Biophys.* **212**(2): 740-753, 1981.

175. YOUNG, N.M., WATSON, D.C. and WILLIAMS, R.E. *Biochem. J.* **222**(1): 41-48, 1984.

176. MATSUMOTO, I. and OSAWA, T. *Biochem.* **13**: 582-588, 1974.

177. CROWLEY, J.F. and GOLDSTEIN, I.J. *FEBS Letters* **130**: 149-152, 1981.

178. KILPATRICK, D.C. and YEOMAN, M.M. *Plant Sci. Lett.* **13**: 35-40, 1978.

179. KILPATRICK, D.C. *Plant Sci. Lett.* **15**: 279-284, 1979.

180. HOREJSI, V. and KOCOUREK, J. *Biochim. Biophys. Acta* **532**(1): 92-97, 1978.

181. KILPATRICK, D.C. and YEOMAN, M.M. *Biochem. J.* **175**(3): 1151-1153, 1978.

182. DESAI, N.N., ALLEN, A.K. and NEUBERGER, A. *Biochem. J.* **197**(2): 345-353, 1981.

183. CROWLEY, J.F., GOLDSTEIN, I.J., ARNARP, J. and LONNGREN, J. *Arch. Biochem. Biophys.* **231**(2): 524-533, 1984.

184. CROWLEY, J.F. and GOLDSTEIN, I.J. *Methods Enzymol.* **83**: 368-373, 1982.

185. HAMMARSTROM, S., MURPHY, L.A., GOLDSTEIN, I.J. and ETZLER, M.E. *Biochem.* **16**(12): 2750-2755, 1977.

186. ETZLER, M.E. and KABAT, E.A. *Biochem.* **9**: 869-877, 1970.

187. CARTER, W.G. and ETZLER, M.E. *J. Biol. Chem.* **250**(7): 2756-2762, 1975.

188. CARTER. W.G. and ETZLER, M, E. *Biochem.* **14**: 2685-2689, 1975.

189. KOCOUREK, J., JAMIESON, G.A., VOTRUBA, T. and HOREJSI, V. *Biochim. Biophys. Acta* **500**(2): 344-360, 1977.

190. ETZLER, M.E., GUPTA, S. and BORREBAECK, C. *J. Biol. Chem.* **256**(5): 2367 2370, 1981.

191. BORREBAECK, C.A.K., LONNERDAL, B. and ETZLER, M.E. *Biochem.* **20**(14): 4119-4122, 1981.

192. ETZLER, M.E. *Methods Enzymol.* **28**: 340-344, 1972.

193. GURAN, A., TICHA, M., FILKA, K. and KOCOUREK, J. *Biochem. J.* **209**(3): 653-657, 1983.

194. CAMMUE, B.P., PEETERS, B. and PEUMANS, W.J. *Biochem. J.* **227**(3): 949-955, 1985.

195. IGLESIAS, J.L., LIS, H. and SHARON, N. *Europ. J. Biochem.* **123**(2): 247-252, 1982

196. GERARDO, P. *Phytochem.* **23**(6): 1229-1232, 1984.

197. HOREJSI, V., TICHA, M., NOVOTNY, J. and KOCOUREK, J. *Biochim. Biophys. Acta* **623**(2): 439-448, 1980.

198. BHATTACHARYYA, L., KUMAR, DAS, P. and SEN, A. *Arch. Biochem. Biophys.* **211**(1): 459-470, 1981.

199. DATTA, T.K. and BASU, P.S. *Biochem. J.* **197**(3): 751-753, 1981.

200. PACAK, F. and KOCOUREK, J. *Biochem. Biophys. Acta* **400**(2): 374-386, 1975.

201. PETRYNIAK, J., PEREIRA, M.E.A. and KABAT, E.A. *Arch. Biochem. Biophys.* **178** (1): 118-134, 1977.

202. BARBIERI, L., FALASCA, A., FRANCESCHI, C., LICASTRO, F., ROSSI, C.A. and STIRPE, F. *Biochem. J.* **215** (3): 433-439, 1983.

203. NSIMBA-LUBAKI, M., PEUMANS, W.J. and CARLIER, A.R. *Biochem. J.* **215**(1): 141-145, 1983.

204. LOTAN, R., CACAN, R., CACAN, M., DEBRAY, H., CARTER, W.G. and SHARON, N. *FEBS Letters* **57**(1): 100-103, 1975.

205. LIS, H., SELA, B.-A., SACHS, L. and SHARON, N. *Biochim. Biophys. Acta* **211**: 582-585, 1970.

206. LIS, H., SHARON, N. and KATCHALSKI, E. *J. Biol. Chem.* **241**(3): 684-689, 1966.

207. WADA, S., PALLANSCH, M.J. and LIENER, I.E. *J. Biol. Chem.* **233**(2): 395-400, 1958.

208. HAMMARSTROM, S., MURPHY, L.A., GOLDSTEIN, I.J. and ETZLER, M.E. *Biochem.* **16**(12); 2750-2755, 1977.

209. LOTAN, R., SIEGELMAN, H.W., LIS, H. and SHARON, N. *J. Biol. Chem.* **249**(4): 1219-1224, 1974.

210. PUEPPKE, S.G. In: Lectins - Biology, Biochemistry, Clinical Biochemistry. Vol. 3. Edited by T.C. Bog-Hansen and G.A. Spengler. Berlin: W. de Gruyter & Co., 1983. pp. 513-520.

211. PEREIRA, M.E. and KABAT, E.A. *Carbohydr. Res.* **37**(1): 89-102, 1974.

212. JAFFE, C.L., EHRLICH-ROGOZINSKI, S., LIS, H. and SHARON, N. *FEBS Letters* **82**(2): 191-196, 1977.

213. GADE, W., JACK, M.A., DAHL, J.B., SCHMIDT, E.L. and WOLD, F. *J. Biol. Chem.* **256**(24): 12905-12910, 1981.

214. GORDON, J.A., BLUMBERG, S., LIS, H. and SHARON, N. *Methods Enzymol.* **28**: 365-368, 1972.

215. LOTAN, R., LIS, H. and SHARON, N. *Biochem. Biophys. Res. Comm.* **62**(1): 144-150, 1975.

216. LIS, H. and SHARON, N. *Methods Enzymol.* **28**: 360-365, 1972.

217. UHLENBRUCK, G. and DAHR, W. *Vox Sang.* **21**: 338-351, 1971.

218. DAHR, W. and UHLENBRUCK, G. *Blut* **22**: 128-132, 1971.

219. MURPHY, L.A. and GOLDSTEIN, I.J. *Methods Enzymol.* **50**: 345-349, 1978.

220. HAYES, C.E. and GOLDSTEIN, I.J. *J. Biol. Chem.* **249**: 1904-1914, 1974.

221. WOOD, C., KABAT, E.A., MURPHY, L.A. and GOLDSTEIN, I.J. *Arch. Biochem. Biophys.* **198**: 1-11, 1979.

222. JUDD, W.J., BECK, M.L., HICKLIN, B.L., SHANKAR, IYER, P.N. and GOLDSTEIN, I.J. *Vox Sang.* **33**: 246-251, 1977.

223. EBISU, S., SHANKAR, IYER, P.N. and GOLDSTEIN, I.J. *Carbohydr. Res.* **61**: 129-138, 1978.

224. EBISU, S. and GOLDSTEIN, I.J. *Methods Enzymol.* **50**: 350-354, 1978.

225. BESSLER, W. and GOLDSTEIN, I.J. *FEBS Letters* **34**: 58-61, 1973

226. MURPHY, L.A. and GOLDSTEIN, I.J. *J. Biol. Chem.* **252**: 4739-4742, 1977.

227. JUDD, W, J, MURPHY, L.A, GOLDSTEIN, I.J. CAMPBELL, L. and NICHOLS, M.E. *Transfusion* **18**: 274-280, 1978.

228. SHANKAR, IYER, P.N., WILKINSON, K.D. and GOLDSTEIN, I.J. *Arch. Biochem. Biophys.* **177**(1): 330-333, 1976.

229. STANLEY, W.S., PETERS, B.P., BLAKE, D.A., YEP, D., CHU, E.H.Y. and GOLDSTEIN, I.J. *Proc. Natl. Acad. Sci. U.S.A.* **76**: 303-307, 1979.

230. MURPHY, L.A. and GOLDSTEIN, I.J. *Biochem.* **18**: 4999-5005, 1979.

231. PARTRIDGE, J., SHANNON, L. and GUMPF, D. *Biochim. Biophys. Acta* **451**: 470-483, 1976.

232. PEUMANS, W.J., STINISSEN, H.M. and CARLIER, A.R. *Biochem. J.* **203**(1): 239-243, 1982.

233. CAMMUE, B., STINISSEN, H.M. and PEUMANS, W.J. *Plant Physiol.* **78**(2): 384-387 1985.

234. MCPHERSON, A. and HOOVER, S. *Biochem. Biophys. Res. Comm.* **89**(2): 713-720, 1979.

235. BARBIERI, L., FALASCA, A., FRANCESCHI, C., LICASTRO, F., ROSSI, C.A. and STIRPE, F. *Biochem. J.* **215**(3): 433-439, 1983.

236. BOYD, W.C. *Vox Sang.* **8**: 1-32, 1963.

237. UHLENBRUCK, G. and DAHR, W. *Vox Sang.* **21**: 338-351, 1971.

238. RENKONEN, K.O. *Ann. Med. Exp. Biol. Fenn.* **26**: 66-72, 1948.

239. MORGAN, W.T.J. and WATKINS, W.M. *Br. J. Exp. Pathol.* **34**: 94-103, 1953.

240. WATKINS, W.M. and MORGAN, W.T.J. *Vox Sang.* 7:129-150, 1962.

241. YEN, S.E., MANSFIELD, J.M. and WALLACE, J.H. *Int. Arch. Allergy Appl. Immunol.* **61**(1): 32-39, 1980.

242. YEN, S.E., MANSFIELD, J.M., LEVINE, R.F. and WALLACE, J.H. *Int. Arch. Allergy Appl. Immunol.* **65**(4): 445-450, 1981.

243. TICHA, M. *et al. Acta Biol. Med. Germ.* **39**(6): 649-655, 1980.

244. KOLBERG, J. *Acta Pathol. Microb. Scand. (C)* **86C**(3): 99-104, 1978.

245. KOLBERG, J., MICHAELSEN, T.E. and SLETTEN, K. In: Lectins - Biology, Biochemistry, Clinical Biochemistry. Edited by T.C. Bog-Hansen. Berlin: W. de Gruyter & Co., 1981. pp. 111-114.

246. GUPTA, B.K., CHATTERJEE-GHOSE, R. and SEN, A. *Arch. Biochem. Biophys.* **201**(1): 137-146, 1980.

247. KOLBERG, J. and SLETTEN, K. *Biochim. Biophys. Acta* **704**(1): 26-30, 1982.

248. HOWARD, I.K., SAGE, H.J., STEIN, M.D., YOUNG, N.M., LEON, M.A. and DYCKES, D.F. *J. Biol. Chem.* **246**(6): 1590-1595, 197.

249. HOWARD, I.K. and SAGE, H.J. *Biochem.* **8**(6): 2436-2441, 1969.

250. TICHA, M., ENTLICHER, G., KOSTIR, J.V. and KOCOUREK, J. *Biochim. Biophys. Acta* **221**: 282-289, 1970.

251. TOYOSHIMA, S., OSAWA, T. and TONOMURA, A. *Biochim. Biophys. Acta* **221**: 514-521, 19

252. PAULOVA, M., TICHA, M., ENTLICHER, G., KOSTIR, J.V. and KOCOUREK, J. *Biochim. Biophys. Acta* **252**: 388-395, 1971.

253. SAGE, H.J. and GREEN, R.W. *Methods Enzymol.* **28**: 332-339, 1972.

254. ZISKA, P., KINDT, A. and FRANZ, H. *Acta Histochem. (Jena)* **71**(1): 29-33, 1982.

255. ZISKA, P. and FRANZ, H. In: Lectins - Biology, Biochemistry, Clinical Biochemistry. Vol. 2. Edited by T.C. Bog-Hansen. Berlin: W. de Gruyter & Co., 1982. pp. 711-720.

256. LAW, I.J. and STRIJDOM, B.W. *Plant Physiol.* **74**(4): 773-778, 1984.

257. NACHBAR, M.A., OPPENHEIM, J.D. and THOMAS, J.0. *J. Biol. Chem.* **255**(5): 2056-2061, 1980.

258. KILPATRICK, D.C. *Biochem. J.* **185**(1): 269-272, 1980.

259. KILPATRICK, D.C. *Prog. Clin. Biol. Res.* **138**: 63-70, 1983.

260. NACHBAR, M.S. and OPPENHEIM, J.D. *Methods Enzymol.* **83**: 363-368, 1982.

261. KAWAGUCHI, T., MATSUMOTO, I. and OSAWA, T. *J. Biol. Chem.* **249**: 2786-2792, 1974.

262. BAUSCH, N.J. and PORETZ, R.D. *Biochem.* **16**(26): 5790-5794, 1977.

263. BIRD, G.W.G. and WINGHAM, J. *Vox Sang.* **24**: 48-57, 1973.

264. SARKAR, M., WU, A.M. and KABAT, E.A. *Arv. Biochem. Biophys.* **209**(1): 204-218, 1981.

265. JONES, J.M. and FELDMAN, J.D. *J. Immunol.* **111**(6): 1765-1770, 1973.

266. ULEVITCH, R.J., JONES, J.M. and FELDMAN, J.D. *Prep. Biochem.* **4**(3): 273-281, 1974.

267. BAUSCH, J.N., RICHEY, J. and PORETZ, R.D. *Biochem.* **20**(9): 2618-2620, 1981.

268. TOMITA, M., KUROKAWA, T., ONOZAKI, K., ICHIKI, N., OSAWA, T. and UKITA, T. *Experientia* **28**: 84-85, 1972.

269. LIN, J.Y., HOU, M.-J. and CHEN, Y.-C. *Toxicon* **16**(6): 653-660, 1978.

270. HOREJSI, V., TICHA, M., NOVOTNY, J. and KOCOUREK, J. *Biochim. Biophys. Acta* **623**(2): 439-448, 1980.

271. LI, S.S.-L. *Experientia* **36**(5): 524-527, 1980.

272. MAZUMDER, T., GAUR, N. and SUROLIA, A. *Eur. J. Biochem.* **113**(3): 463-470, 1981.

273. GHOSH, B.N. DASGUPTA, B. and SIRCAR, P.K. *Indian J. Exp. Biol.* **19**(3): 253-255, 1981.

274 HAPNER, K. D. and ROBBINS, J. E. *Biochim. Biophys. Acta* **580**(1): 186-197, 1979.

275. KOUCHALAKOS, R.N. and HAPNER, K.D. *Biochim. Biophys. Acta* **787**(3): 237-243, 1984.

276. NAMEN, A.E. and HAPNER, K.D. *Biochim. Biophys. Acta* **580**(1): 198-209, 1979.

277. HOREJSI, V., CHALOUPECKA, 0. and KOCOUREK, J. *Biochim. Biophys. Acta* **539**(3): 287-293, 1978.

278. TSUDA, M. *J. Biochem.* **86**(5): 1451-1461, 1979.

279. TABARY, F., BALANDREAU, J. and BOURRILLON, R. *Biochem. Biophys. Res. Commun.* **119**(2): 549-555, 1984.

280. SHEN, Z.-W., SUN, C., ZHU, Z., TANG, X.-H. and SHEN, R.-J. *Can. J. Biochem. Cell Biol.* **62**(10): 1027-1032, 1984.

281. MEADE, N.A., STAAT, R.H., LANGLEY, S.D. and DOYLE, R.J. *Carbohydr. Res.* **78**(2): 349-363, 1980.

282. GALBRAITH, W. and GOLDSTEIN, I.J. *Biochem.* **11**(21): 3976-3984, 1972.

283. GOULD, N.R. and SCHEIGBERG, S.L. *Arch. Biochem. Biophys.* **137**: 1-11, 1970.

284. GALBRAITH, W. and GOLDSTEIN, I.J. *FEBS Letters* **9**(4): 197-201, 1970.

285. BESSLER, W. and GOLDSTEIN, I.J. *Arch. Biochem. Biophys.* **165**: 444-445, 1974.

286. RUDDON, R.W., WEISENTHAL, L.M., LUNDEEN, D.E., BESSLER, W. and GOLDSTEIN, I.J. *Proc. Natl. Acad. Sci. U.S.A.* **71**(5): 1848-1851, 1974.

287. PANDOLFINO, E.R. and MAGNUSON, J.A. *J. Biol. Chem.* **255**(3): 870-873, 1980.

288. GALBRAITH, W. and GOLDSTEIN, I.J. *Methods Enzymol.* **28**: 318-322, 1972.

289. KORNFELD, R., GREGORY, W.T. and KORNFELD, S.A. *Methods Enzymol.* **28**: 344-349, 1972

290. PUSZTAI, A. and STEWART, J.C. *Biochim. Biophys. Acta* **536**(1): 38-49, 1978.

291. ANDREWS, A.T. *Biochem J.* **139**(2): 421-429, 1974.

292. WEBER, T.H., ARO, H. and NORDMAN, C.T. *Biochim. Biophys. Acta* **263**: 94-105, 1972.

293. WEBER, T.H. *Scand. J. Clin. Lab. Invest.* **24**(suppl. 111): 1-80, 1969.

294. YACHNIN, A. and SVENSON, R.H. *Immunol.* **22**: 871-883, 1972.

295. FELSTED, R.L., EGORIN, M.J., LEAVITT, R.D. and BACHUR, N.R. *J. Biol. Chem.* **252**(9): 2967-2971, 1977.

296. KORNFELD, R. and KORNFELD, S. *J. Biol. Chem.* **245**(10): 2536-2545, 1970.

297. RIGAS, D.A. and OSGOOD, E.E. *J. Biol. Chem* **212**: 607-615, 1955.

298. RASANEN, V., WEBER, T.H. and GRASBECK, R. *Eur. J. Biochem.* **38**: 193-200, 1973

299. LEAVITT, R.D., FELSTED, R.L. and BACHUR, N.R. *J. Biol. Chem.* **252**(9): 2961-2966, 1977.

300. MILLER, J.B., NOYES, C., HEINRIKSON, R., KINGDON, H.S. and YACHNIN, S. *J. Exp. Med.* **138**: 939-951, 1973.

301. MILLER, J.B., HSU, R., HEINRIKSON, R. and YACHNIN, S. *Proc. Natl. Acad. Sci.* **72**(4) 1388-1391, 1975.

302. BORBERG, H., WOODRUFF, J., HIRSCHHORN, R., GESNER, B., MIESCHER, P. and SILBER, R. *Science* **154**: 1019-1020, 1966.

303. BORBERG, H., YESNER, I., GESNER, B. and SILBER, S. *Blood* **31**(6): 747-757, 1968.

304. DAHLGREN, K. and PORATH, J. *Arch. Biochem. Biophys.* **137**: 306-314, 1970.

305. BIRD, G.W.G. and WINGHAM, J. *Experientia* **26**: 1257-1258, 1970.

306. WAXDAL, M.J. *Biochem.* **13**(18): 3671-3677, 1974.

307. YOKOYAMA, K., TERAO, T. and OSAWA, T. *Biochim. Biophys. Acta* **538**: 384-396, 1978.

308. CHESSIN, L.N., BORJESON, J., WELSH, P.D., DOUGLAS, S.D. and COOPER, H.L. *J. Exp. Med.* **124**: 873-884, 1966.

309. YOKOYAMA, K., YANO, 0., TERAO, T. and OSAWA, T. *Biochim. Biophys. Acta* **427**: 443-452, 1976.

310. WAXDAL, M.J. and BASHAM, T.Y. *Nature* **251**: 163-164, 1974.

311. FARNES, P., BARKER, B.E., BROWNHILL, L.E. and FANGER, H. *Lancet* **2**: 1100-1101, 1964

312. WAXDAL, M.J. *Methods Enzymol.* **50**: 354-361, 1978.

313. TROWBRIDGE, I.S. *J. Biol. Chem.* **249**(18): 6004-6012, 1974.

314. ENTLICHER, G., TICHA, M., KOSTIR, J.V. and KOCOUREK, J. *Experientia* **25**: 17-19, 1969.

315. ENTLICHER, G., KOSTIR, J.V. and KOCOUREK, J. *Biochim. Biophys. Acta* **221**: 272-281, 1970.

316. VAN WAUWE, J.P., LOONTIENS, F.G. and DE BRUYNE, C.K. *Biochem. Biophys. Acta* **379**(2): 456-461, 1975.

317. KORNFELD, K., REITMAN, M.L. and KORNFELD, R. *J. Biol. Chem.* **256**(13): 6633 6640, 1981.

318. PAULOVA, M., ENTLICHER, G., TICHA, M., KOSTIR, J.V. and KOCOUREK, J. *Biochim. Biophys. Acta* **237**: 513-518, 1971.

319. VAN DRIESSCHE, E., SMETS, G., DEJAEGERE, R. and KANAREK, L. In: Lectins - Biology, Biochemistry, Clinical Biochemistry. Vol.2. Edited by T.C. Bog-Hansen. Berlin: W. de Gruyter & Co., 1982. pp. 729-742.

320. PUEPPKE, S.G. *Biochim. Biophys. Acta* **581**(1): 63-70, 1979.

321. APPUKUTTAN, P.S. and BASU, D. *Anal. Biochem.* **113**(2): 253-255, 1981.

322. KORTT, A. *Eur. J. Biochem.* **138**(3): 519-525, 1984.

323. KORTT, A. *Arch. Biochem. Biophys.* **236**(2): 544-554, 1985.

324. KROLICK, K.A., VILLEMEZ, C., ISAKSON, P., UHR, J.W. and VITETTA, E.S. *Proc. Natl. Acad. Sci. U.S.A.* **77**(9): 5419-5423, 1980.

325. NICOLSON, G.L. and BLAUSTEIN, J. *Biochim. Biophys. Acta* **266**: 543-547, 1972.

326. NICOLSON, G.L., BLAUSTEIN, J. and ETZLER, M.E. *Biochem.* **13**(1): 196-204, 1974.

327. WEI, C.H. and KOH, C. *J. Mol. Biol.* **123**: 707-711, 1978.

328. LIN, T.S. and LI, S.L. *Eur. J. Biochem.* **105**(3): 453-459, 1980.

329. TOMITA, M., KUROKAWA, T., ONOZAKI, K., ICHIKI, N., OSAWA, T. and UKITA, T. *Experientia* **28**: 84-85, 1972.

330. OLSNES, S., SALTVEDT, E. and PIHL, A. *J. Biol. Chem.* **249**(3): 803-810, 1974.

331. BOURRILLON, R. and FONT, J. *Biochim. Biophys. Acta* **154**: 28-39, 1968.

332. MARKOWITZ, H. *Fed. Proc.* **30**: 2772 (Immunology), 1971.

333. LEMONNIER, M., GOUSSAULT, Y. and BOURRILLON, R. *Carbohydr. Res.* **24**: 323-331, 1972.

334. SHARIF, A., BROCHIER, J. and BOURRILLON, R. *Cell Immunol.* **31**(2): 302-310, 1977.

335. HOREJSI. V., HASKOVEC, C. and KOCOUREK, J. *Biochim. Biophys. Acta* **532**(1): 98-104, 1978.

336. MCPHERSON, A. and HOOVER, S. *Biochem. Biophys. Res. Commun.* **89**(2): 713-720, 1979.

337. BIRD, G.W.G. and WINGHAM, J. *Vox Sang.* **26**: 163-166, 1974.

338. BIRD, G.W.G. and WINGHAM, J. *Vox Sang.* **30**(3): 217-219, 1976.

339. BIRD, G.W.G. and WINGHAM, J. *Vox Sang.* **32**(2): 121-122, 1977.

340. BIRD, G.W.G. and WINGHAM, J. *Vox Sang.* **26**: 163-166, 1974.

341. KOURTEVA, B.T., MANOLOVA, V.R. and MITOVA, D.K. *Transfusion* **17**(3): 272-276, 1977.

342. BIRD, G.W.G. and WINGHAM, J. *Vox Sang.* **30**(3): 217-219, 1976.

343. BIRD, G.W.G. and WINGHAM, J. *Vox Sang.* **32**(2): 121-122, 1977.

344. Lee, L.T., FRANK, S., DE JONGH, D.S. and HOWE, C. *Blood* **58**(6): 1228-1231, 1981.

345. BROEKAERT, W. F. NSIMBA-LUBAKI, M. PEETERS, B. and PEUMANS, W.J. *Biochem. J.* **221**(1): 163-169, 1984.

346. PEUMANS, W.J., STINISSEN, H.M. and CARLIER, A.R. *Biochem. J.* **203**(1): 239-243,1982

347. ALLEN, A.K. and NEUBERGER, A. *Biochem. J.* **135**: 307-314, 1973.

348. MARINKOVITCH, V.A. *J. Immunol.* **93**: 732-741, 1964.

349. DELMOTTE, F., KIEDA, C. and MONSIGNY, M. *FEBS Letters* **53**(3): 324-330, 1975.

350. DESAI, N.N. and ALLEN, A.K. *Anal. Biochem.* **93**: 88-90, 1979.

351. ALLEN, A.K. and NEUBERGER, A. *Methods Enzymol.* **50**: 340-345, 1978.

352. KILPATRICK, D.C. *Biochem. J.* **191**(1): 273-275, 1980.

353. MATSUMOTO, I., JIMBO, A., MIZUNO, Y., SENO, N. and JEANLOZ, R.W. *J. Biol. Chem.* **258**(5): 2886-2891, 1983.

354. VAN DRIESSCHE, E., BEECKMANS, S., DEJAEGERE, R. and KANAREK, L. In: Lectins Biology, Biochemistry, Clinical Biochemistry. Vol. 3. Edited by T.C. Bog-Hansen and G.A. Spengler. Berlin: W. de Gruyter & Co., 1983. pp. 629-638.

355. IRIMURA, T., KAWAGUCHI, T., TERAO, T. and OSAWA, T. *Carbohydr. Res.* **39**: 317-327, 1975.

356. PORETZ, R.D. *Methods Enzymol.* **28**: 349-354, 1972.

357. MORGAN, W.T.J. and WATKINS, W.M. *Brit. J. Exp. Pathol.* **34**: 94-103, 1953.

358. PORETZ, R.D. and BARTH, R.F. *Immunol.* **31**: 187-194, 1976.

359. PORETZ, R.D., RISS, H., TIMBERLAKE, J.W. and CHIEN, S.-M. *Biochem.* **13**(2): 250-256, 1974.

360. CHIEN, S.-M., SINGLA, S. and PORETZ, R.D. *J. Immunol. Methods* **8**(1-2): 169-174, 19

361. TIMBERLAKE, J.W., WONG, R.B.C. and PORETZ, R.D. *Prep. Biochem.* **10**(2): 173-190, 1980.

362. NEUCERE, J.N. *J. Agric. Food Chem.* **30**(3): 603-604, 1982.

363. PEREIRA, M.E.A. and KABAT, E.A. *Ann. N.Y. Acad. Sci.* **234**: 301-305, 1974.

364. YARIV, J., KALB, A.J. and KATCHALSKI, E. *Nature* **215**: 890-891, 1967.

365. KALB, A.J. *Biochim. Biophys. Acta* **168**: 532-536, 1968.

366. MORGAN, W.T.J. and WATKINS, W.M. *Brit. J. Exp. Pathol.* **34**: 94-103, 1953.

367. SPRINGER, G.F. and WILLIAMSON, P. *Biochem. J.* **85**: 282-291, 1962.

368. YARIV, J., KALB, A.J. and BLUMBERG, S. *Methods Enzymol.* **28**: 356, 1972.

369. PEREIRA, M.E. and KABAT, E.A. *Biochem.* **13**(15): 3184-3192, 1974.

370. GOLDSTEIN, I.J., HAMMARSTROM, S. and SUNDBLAD, G. *Biochim. Biophys. Acta* **405**: 53-61, 1975.

371. NAGATA, Y. and BURGER, M.M. *J. Biol. Chem.* **249**(10): 3116-3122, 1974

372. ALLEN, A.K., NEUBERGER, A. and SHARON, N. *Biochem. J.* **131**: 155-162, 1973.

373. RICE, R.H. and ETZLER, M.E. *Biochem. Biophys. Res. Commun.* **59**(1): 414-420, 1974.

374. RICE, R.H. and ETZLER, M.E. *Biochem.* **14**(18): 4093-4099, 1975.

375. NAGATA, Y. and BURGER, M.M. *J. Biol. Chem.* **247**(7): 2248-2250, 1972.

376. LEVINE, D., KAPLAN, M.J. and GREENAWAY, P.J. *Biochem. J.* **129**: 847-856, 1972.

377. SHAPER, J.H., BARKER, R. and HILL, R.L. *Anal. Biochem.* **53**: 564-570, 1973.

378. MONSIGNY, M., SENE, C., OBRENOVITCH, A., ROCHE, A.-C., DELMOTTE, F. and BOSCHETTI, E. *Eur. J. Biochem.* **98**: 39-45, 1979.

379. MARCHESI, V.T. *Methods Enzymol.* **28**: 354-356, 1972.

380. FROST, R.G., REITHERMAN, R.W., MILLER, A.L. and O'BRIEN, J.S. *Anal. Biochem.* **69**: 170-179, 1975.

381. MATSUMOTO, I. and OSAWA, T. *Biochim. Biophys. Acta* **194**: 180-189, 1969.

382. MATSUMOTO, I. and OSAWA, T. *Arch. Biochem. Biophys.* **140**: 484-491, 1970.

383. OSAWA, T. and MATSUMOTO, I. *Methods Enzymol.* **28** iPtB: 323-327, 1972.

384. PEREIRA, M.A., GRUEZO, F. and KABAT, E.A. *Arch. Biochim. Biophys.***194**: 511-525, 1979.

385. HOREJSI, V. *Biochim. Biophys. Acta* **577**(2): 389-393, 1979.

386. ONOZAKI, K., HOMMA, Y. and HASHIMOTO, T. *Experientia* **35**(12): 1556-1557, 1979.

387. ASPBERG, K., HOLMAN, H. and PORATH, J. *Biochim. Biophys. Acta* **160**: 116-117, 1968.

388. SUNDBERG, L., PORATH, J. and ASPBERG, K. *Biochim. Biophys. Acta* **221**: 394-395, 1970.

389. RUDIGER, H. *Eur. J. Biochem.* **72**(2): 317-322, 1977.

390. BAUMANN, C., RUDIGER, H. and STROSBERG, A.D. *FEBS Letters* **102**(2): 216-218, 1979.

391. KARHI, K.K. and GAHMBERG, C.G. *Biochim. Biophys. Acta* **622**(2): 337-343, 1980.

392. BAUMANN, C.M., STROSBERG, A.D. and RUDIGER, H. *Eur. J. Biochem.* **122**(1): 105-110, 1982.

393. BAUMANN, C.M., RUDIGER, H. and STROSBERG, A.D. In: Lectins - Biology, Biochemistry, Clinical Biochemistry. Vol.1. Edited by T.C. Bog-Hansen. Berlin: W. de Gruyter & Co., 1981. pp. 93-101.

394. FORNSTEDT, N. and PORATH, J. *FEBS Letters* **57**(2): 187-191, 1975.

395. MATSUMOTO, I., UEHARA, Y., JIMBO, A. and SENO, A. *J. Biochem.* **93**: 763-769, 1983.

396. HEMPERLY, J.J., HOPP, T.P., BECKER, J.W. and CUNNINGHAM, B.A. *J. Biol. Chem.* **254**(14): 6803-6810, 1979.

397. ALLEN, A.K., DESAI, N.N. and NEUBERGER, A. *Biochem. J.* **155**(1): 127-135, 1976.

398. WANG, J.L., BECKER, J.W., REEKE, G.N. Jr. and EDELMAN, G.M. *J. Mol. Biol.* **88**(1): 259-262, 1974.

399. ALLEN, H.J. and JOHNSON, E.A.Z. *Biochim. Biophys. Acta* **444**(2): 374-385, 1976.

400. ALLEN, A.K., DESAI, N.N. and NEUBERGER, A. *Methods Enzymol.* **50**: 335-339, 1978.

401. OTTENSOOSER, F. and SILBERSCHMIDT, K. *Nature* **172**: 914, 1953.

402. PRIGENT, M.J. and BOURRILLON, R. *Biochim. Biophys. Acta* **420**(1): 112-121, 1976.

403. SPRINGER, G.F., NAGAI, Y. and TEGTMEYER, H. *Biochem.* **5**(10): 3254-3272, 1966.

404. UHLENBRUCK, G. and DAHR, W. *Vox Sang.* **21**: 338-351, 1971.

405. FALASCA, A., FRANCESCHI, C., ROSSI, C.A. and STIRPE, F. *Biochim. Biophys. Acta* **577**(1): 71-81, 1979.

406. GEBAUER, B., SCHILTZ, E., SCHIMPL, A. and RUDIGER, H. *Hoppe Seylers Z. Physiol. Chem.* **360**(12): 1727-1735, 1979.

407. FALASCA, A., HRELIA, S., ROSSI, C.A., LICASTRO, F., CHIRICOLO, M., STIRPE, F. and FRANCESCHI, C. In: Lectins - Biology, Biochemistry, Clinical Biochemistry. Vol.2. Edited by T.C. Bog-Hansen. Berlin: W. de Gruyter & Co., 1982. pp. 759-770.

408. MOON, G.J. and WIENER, A.S. *Vox Sang.* **26**: 167-170, 1974.

409. TOLLEFSEN, C.E. and KORNFELD, R. *J. Biol. Chem.* **258**(8): 5165-5171, 1983.

410. KIMURA, A., WIGZELL, H., HOLMQUIST, G., ERSSON, B. and CARLSSON, P. *J. Exp. Med.* **149**: 473-484, 1979.

411. GRUBHOFFER, L., TICHA, M. and KOCOUREK, J. *Biochem. J.* **195**(3): 623-626, 1981.

412. HANKINS, C.N. and SHANNON, L.M. *J. Biol. Chem.* **253**(21): 7791-7797, 1978.

413. STIRPE, F., LEGG, R.F., ONYON, L.J., ZISKA, P. and FRANZ, H. *Biochem. J.* **190**: 843-845, 1980.

414. SEHRT, I. and LUTHER, P. In: Lectins - Biology, Biochemistry, Clinical Biochemistry. Vol.2. Edited by T.C. Bog-Hansen. Berlin: W. de Gruyter & Co., 1982. pp. 45-46.

415. LUTHER, P. *Acta Biol. Med. Ger.* **35**: 123-126, 1976.

416. LUTHER, P. *Acta Biol. Med Ger* **36**: 119-125, 1977.

417. ZISKA, P., FRANZ, H. and KINDT, A. *Experientia* **34**(1): 123-124, 1978.

418. LUTHER, P., THEISE, H., CHATTERJEE, B-, KARDUCK, D- and UHLENBRUCK, G. *Int. J. Biochem.* **11**(5): 429-435, 1980.

419. FRANZ, H. ZISKA, P. and KINDT, A. *Biochem. J.* **195**(2): 481-484, 1981.

420. KUROKAWA, T., TSUDA, M. and SUGINO, Y. *J. Biol. Chem.* **251**(18): 5686-5693, 1976.

421. TOYOSHIMA, S., AKIYAMA, Y., NAKANO, K., TONOMURA, A. and OSAWA, T. *Biochem.* **10**(24) 4457-4463, 1971.

422. BARKER, B.E. and FARNES, P. *Nature (London)* **215**: 659-660, 1967.

423. TOYOSHIMA, S. and OSAWA, T. *J. Biol. Chem.* **250**(5): 1655-1660, 1975.

424. KALADAS, P.M. and PORETZ, R.D. *Biochem.* **18**(22): 4806-4812, 1979.

425. OSAWA, T. and TOYOSHIMA, S. *Methods Enzymol.* **28**: 328-331, 1972.

426. MORGAN, W.T.J. and WATKINS, W.M. *Brit. J. Exp. Pathol.* **34**: 94-103, 1953.

427. BOYD, W.C, .and SHAPLEIGH, E. *Science* **119**: 419, 1954.

428. BOYD, W.C. and SHAPLEIGH, E. *J. immunol.* **73**: 226-231, 1954.

429. BIRD, G.W.G. *Vox Sang.* **9**: 748-749, 1964.

430. BOYD, W.C. *Ann. N.Y. Acad. Sci.* **169**: 168-190, 1970.

431. GORDON, J.A. SHARON, N. and LIS, H. *Biochim. Biophys. Acta* **264**: 387-391, 1972.

432. CHOWDHURI, S. and CHATTERJEE, P.C. *Indian J. Med. Res.* **61**(10): 1478-1484, 1973.

433. BOYD, W.C. *Science* **186**: 846, 1974.

434. BOYD, W.C. *Ann. N.Y. Acad. Sci.* **234**: 396-408, 1974.

435. LEVINE, P. *Ann. N.Y. Acad. Sci.* **234**: 306-309, 1974.

436. KHANNA, A.K. In: Lectins - Biology, Biochemistry, Clinical Biochemistry.Vol.2. Edited by T.C. Bog-Hansen. Berlin: W. de Gruyter & Co., 1982. pp. 70-709.

437. TSUJI, T. and OSAWA, T. *Tanpakushitsu Kakusan Koso* **28**(2): 118-131, 1983.

438. RUTISHAUSER, U. and SACHS, L. *J. Cell Biol.* **65**: 247-257, 1975.

439. KORNFELD, S. and KORNFELD, R. In: The Glycoconjugates Vol.2 Mammalian Glycoprotein, Glycolipids and Proteoglycans. Edited by M.I. Horowitz and W. Pigman. New York: Academic Press, 1978. pp. 437-449.

440. DULANEY, J.T. *Mol. Cell. Biochem.* **21**(1): 43-63, 1979.

441. ALFORD, R.H. *J. Immunol.* **104**(3): 698-703, 1970.

442. CUATRECASAS, P. and TELL, G.P.E. *Proc. Nat. Acad. Sci. U.S.A.* **70**(2): 485-489, 1973.

443. EWART, R.B.L., KORNFELD, S. and KIPNIS, D.M. *Diabetes* **24**: 705-714, 1975.

444. BESANCON, F. and ANKEL, H. *Nature* **250**: 784-786, 1974.

445. MIRELMAN, D., GALUN, E., SHARON, N. and LOTAN, R. *Nature* **256**: 414-416, 1975.

446. CHESSIN, L.N., BORJESON, J., WELSH, P.D., DOUGLAS, S.D. and COOPER, H.L. *J. Exp. Med.* **124**: 873-884, 1966.

447. GREAVES, M.F. and BAUMINGER, S. *Nature New Biology* **235**: 67-70, 1972.

448. NOVOGRODSKY, A. and KATCHALSKI, E. *Biochim. Biophys. Acta* **228**: 579-583, 1971.

449. PARKER, C.W. *Adv. Cycl. Nucl. Res.* **9**: 647-660, 1978.

450. EGAN, H.S., REEDER, W.J. and EKSTEDT, R.D. *J. Immunol.* **112**(1): 63-69, 1974.

451. NICOLSON, G.L. In: Biology and Chemistry of Eucaryotic Cell Surfaces (Miami Winter Symposia Vol. 7). Edited by E.Y.C. Lee and E.E. Smith. New York: Academic Press, 1974. pp. 103-124.

452. WRIGHT, T.C., UKENA, T.E. and KARNOVSKY, M.J. *Prog. Exp. Tumor Res.* **22**: 1-27, 1978. **22**: 1-27, 1978.

453. NICOLSON, G.L. *Biochim. Biophys. Acta* **458**: 1-72, 1976.

454. INBAR, M. and SACHS, L. *Nature* **223**: 710-712, 1969.

455. BURGER, M.M. and GOLDBERG, A.R. *Proc. Natl. Acad. Sci.* **57**: 359-366, 1967.

456. NICOLSON, G.L. *Nature New Biology* **233**: 244-246, 1971:

457. RAPIN, A.M.C. and BURGER, M.M. *Adv. Can. Res.* **20**: 1-91, 1974.

458. HUGHES, R.C. Membrane. Glycoproteins - A. Review of Structure and Function. London: Butterworth & Co. Ltd., 1976. pp. 135-151.

459. KORNFELD, S., ADAIR, W.L., GOTTLIEB, C. and KORNFELD, R. In: Biology and Chemistry of Eucaryotic Cell Surfaces (Miami Winter Symposia Vol. 7). Edited by E.Y.C. Lee and E.E. Smith. New York: Academic Press, 1974.

460. STIRPE, F., LEGG, R.F., ONYON, L.J., ZISKA, P. and FRANZ, H. *Biochem. J.* **190**: 843-845, 1980.

461. JUDD, W.J. *Transfusion* **19**(6): 768-772, 1979.

462. BOYD, W.C. and SHAPLEIGH, E. *J. Lab. & Clin. Med.* **44**(2): 235-237, 1954.

463. BOLDT, D.H. and LYONS, R.D. *Cell. Immunol.* **43**: 82-93, 1979.

464. REISNER, Y., RAVID, A. and SHARON, N. *Biochem. Biophys. Res. Comm.* **72**(4): 1585-1591, 1976.

465. KIMURA, A., WIGZELL, H., HOLMQUIST, G., ERSSON, B. and CARLSSON, P. *J. Exp. Med.* **149**: 473-484, 1979.

466. SCHNEBLI, H.P. and DUKOR, P. *Eur. J. Immunol.* **2**: 607-609, 1972.

467. COGGI, G., DELL'ORTO, P., BONOLDI, E., DOI, P. and VIALE, G. In: Lectins Biology, Biochemistry, Clinical Biochemistry. Vol. 3. Edited by T.C. Bog-Hansen and G.A. Spengler. Berlin: W. de Gruyter & Co., 1983. pp. 87-103.

468. COOPER, H.S. *Hum. Pathol.* **15**(10): 904-906, 1984.

469. LOUIS, C.J., SZTYNDA, T., CHENG, Z.-M. and WYLLIE, R.G. *Cancer* **52**: 1244-1250, 1983.

470. RAEDLER, A. and RAEDLER, E. *J. Can. Res. Clin. Oncol.* **109**: 245-251, 1985.

471. REANO, A., FAURE, M., JACQUES, Y. and REICHERT, U. . *Brit. J. Derm.* **107** (Suppl 23): 143-146, 1982.

472. ALROY, J., ORGAD, U., UCCI, A.A. and PEREIRA, M.E.A. *J. Histochem. Cytochem.* **32**(12): 1280-1284, 1984.

473. S.V., Editorials. *J.A.M.A.* **213**(2): 289-290, 1970.

474 LIN, J.-Y. LI, J.-S. and TUNG, T.-C. *J. Nat. Can. Inst.* **66**(3): 523-528, 1981.

475. STODDART, R.W. and HERBERTSON, B.M. *Biochem. Soc. Trans.* **5**: 233-235, 1977.

476. LOTAN, R. and NICOLSON, G.L. *Biochim. Biophys. Acta* **559**: 329-376, 1979.

477. SHARON, N. In: Cell Separation: Methods and Selected Applications. Vol. 3. Edited by T.G. Pretlow and T.P. Pretlow. Orlando, Fla.: Academic Press, Inc., 1984. pp. 13-52.

478. MCCOY, J.P. Jr., VARANI, J. and GOLDSTEIN, I.J. *Anal. Biochem.* **130**: 437-444, 1983.

479. STEINEMANN, A. and STRYER, L. *Biochem.* **12**(8): 1499-1502, 1973.

480. BOG-HANSEN, T.C., BJERRUM, 0.J. and BROGREN, C.-H. *Anal. Biochem.* **81**: 78-87, 1977.

481. OWEN, P., OPPENHEIM, J.D., NACHBAR, M.S. and KESSLER, R.E. *Anal. Biochem.* **80**: 446-457, 1977.

482. HOREJSI, V., TICHA, M. and TRNKA, T. *Anal. Biochem.* **137**: 170-174, 1984.

483. STANLEY, P. In: The Biochemistry of Glycoproteins and Proteoglycans. Edited by W.J. Lennarz. New York: Plenum Press, 1980. pp. 161-189.

484. SHARON, N. and LIS, H. In: Methods in Membrane Biology. Vol. 3. Plasma Membranes. Edited by E.D. Korn. New York: Plenum Press, 1975. pp. 147-200.

485. PORETZ, R.D. In: Carbohydrate-Protein Interaction. ACS, Symposium Series 88. Edited by I.J. Goldstein. Washington, D,C.: American Chemical Society, 1979. pp. 56-66.

486. LOTAN, R. *Scan. Electron Micros.* (3): 549-564, 1979.

487. ROTH, J. *Acta Histochem.* 22(Suppl.): 113-121, 1980.

488. BIRD, G.W.G. *Ann. N.Y. Acad. Sci.* 234: 129-138, 1974.

489. VIRTANEN, I., LEHTO, V.-P. and AULA, P. In: Lectins - Biology, Biochemistry, Clinical Biochemistry. Vol. 1. Edited by T.C. Bog-Hansen. Berlin: W. de Gruyter & Co., 1981. pp. 215-220.

490. WINQVIST, L., ERIKSSON, L., DALLNER, G. and ERSSON, B. *Biochem. Biophys. Res. Comm.* 68(3): 1020-1026, 1976.

491. MORGAN, A.C. Jr., WOODHOUSE, C.S., KNOST, J.A., ABRAM, P.G., CLARKE, G.C., ARTHUR, L.O., MCINTYRE, R., OCHS, J.J., FOON, K.A. and OLDHAM, R.K. *Hybridoma* 3(3): 233-245, 1984.

492. PHILLIPSON, 0.T. and GRIFFITHS, A.C. *Brain Res.* 265: 199-207, 1983.

493. PISTOLE, T.G. . *Ann. Rev. Microbiol.* 35: 85-112, 1981.

494. KAHN, L.D. *Physiol. Chem Phys.* 14: 3-7 1982.

495. MARUYAMA, H.B. *Biochim. Biophys. Acta* 274: 499-504, 1972

496. ARCHIBALD, A.R. and COAPES, H.E. *Biochem. J.* 123: 665-667, 1971.

497. ROLLA, G. *Archs. Oral Biol.* 16: 967-969, 1971.

498. HAMADA, S. GILL, K. and SLADE, H.D. *Infect. Immun.* 18(3): 708-716, 1977.

499. ABAAS, S. *Acta Path. Microbiol. Immunol. Scand.* 93(B): 15-20, 1985.

500. WAGNER, M. *Immunobiol.* 156: 57-64, 1979.

501. GRAY, B.M., DILLON, H.C. Jr. and PRITCHARD, D.G. *J. Immunol. Meth.* 72: 269-277, 1984

502. SCHIEFER, H.-G., GERHARDT, U., BRUNNER, H. and KRUPE, M. *J. Bact.* 120(1): 81-88, 1974

503. SCHIEFER, H.-G., GERHARDT, U., BRUNNER, H. and KRUPE, M. *Hoppe Seylers Z. Phys Chem.* 355: 1248, 1974.

504. KAHANE, I. and TULLY, J.G. *J. Bact.* 128(1): 1-7, 1976.

505. AMAR, A., KAHANE, I., ROTTEM, S. and RAZIN, S. *Microbios* 24: 93-102, 1979.

506. SODERLUND, G. and KIHLSTROM, E. *Infect. Immun.* 42(3): 930-935, 1983.

507. BOSE, S.K., SMITH, G.B. and PAUL, R.G. *Infect. Immun.* 40(3): 1060-1067, 1983.

508. LEVY, N.J. *Infect. Immun.* 25(3): 946-953, 1979.

509. GOLDSTEIN, I.J. and STAUB, A.M. *Immunochem.* 7: 315-319, 1970.

510. ALLEN, P.Z. *Infect. Immun.* 47(1): 90-93, 1985.

511. REEDER, W.J. and EKSTEDT, R.D. *J. Immunol.* 106(2): 334-340, 1971.

512. GOLDSTEIN, I.J. and MISAKI, A. *J. Bact.* 103(2): 422-425, 1970.

513. DOYLE, R.J. and BIRDSELL, D.C. *J. Bact.* 109(2): 652-658, 1972.

514. DOYLE, R.J., NEDJAT-HAIEM, F., MILLER, R.D. and KELLER, K.F. *J. Clin. Micro.* 15(5): 973-975, 1982.

515. CORBEL, M.J. and BREWER, R.A. *Vet. Rec.* 112: 202, 1983.

516. MULLICK, A. *Ind. J. Med. Res.* 60: 840-843, 1972.

517. WONG, K.H., SKELTON, S.K. and FEELEY, J.C. *J. Clin. Micro.* 22(1): 134-135, 1985.

518. SMALLEY, D.L. and BRADLEY, M.E. *Can. J. Microbiol.* 29: 619-621, 1983.

519. WADSTROM, T., TRUST, T.J. and BROOKS, D.E. In: Lectins - Biology Biochemistry, Clinical Biochemistry. Vol. 3 Edited by T.C. Bog-Hansen and G.A. Spengler. Berlin: W. de Gruyter & Co., 1983. pp. 479-494.

520. TOSHIYUKI, K. and KUNIO, 0. *Tanpakushitsu Kakusan Koso* 28(2): 132-145, 1983.

521. GILBOA-GARBER, N. In: Lectins - Biology, Biochemistry, Clinical Biochemistry. Vol. 3. Edited by T.C. Bog-Hansen and G.A. Spengler. Berlin: W. de Gruyter & Co., 1983. pp. 495-502.

522. GILBOA-GARBER, N. *Methods Enzymol.* 83: 378-385, 1982.

523. ESHADAT, Y. and SHARON, N. *Methods Enzymol.* 83: 386-391, 1982.

524. WADSTROM, T., FARIS, A., LINDAHL, M., LONNERDAHL, B. and ERSSON, B. In: Lectins - Biology, Biochemistry, Clinical Biochemistry. Vol. 3. Edited by T.C. Bog-Hansen and G.A. Spengler. Berlin: W. de Gruyter & Co., 1983. pp. 503-510.

525. RICHARDS, R.L., MOSS, J., ALVING, C.R., FISHMAN, P.H. and BRADY, R.0. *Proc. Natl. Acad. Sci.* 76(4): 1673-1676, 1979.

526. FIRON, N., OFEK, I. and SHARON, N. *Biochem. Biophys. Res. Comm.* 105(4): 1426-1432, 1982.

527. FIRON, N., OFEK, I. and SHARON, N. *Infect. Immun.* 43(3): 1088-1090, 1984.

528. FIRON, N., OFEK, I. and SHARON, N. *Carbohydr. Res.* 120: 235-249, 1983.

529. ESHDAT, Y., OFEK, I., YASHOUV-GAN, Y., SHARON, N. and MIRELMAN, D. *Biochem. Biophys. Res. Comm.* 85(4): 1551-1559, 1978.

530. ESHDAT, Y. and SHARON, N. In: Lectins - Biology, Biochemistry, Clinical Biochemistry. Vol. 3. Edited by T.C. Bog-Hansen and G.A. Spengler. Berlin: W. de Gruyter & Co., 1983. pp. 667-675.

531. ESHDAT, Y. and SHARON, N. *Bio. Cell* **51**: 259-266, 1984.

532. OFEK, I., GOLDHAR, J., ESHDAT, Y. and SHARON, N. *Scand. J. Infect. Dis. Suppl.* **33**: 61-67, 1982.

533. OFEK, I., BEACHEY, E.H. and SHARON, N. *TIBS* pp. 159-160, 1978.

534. ELAD, Y., BARAK, R. and CHET, I. *J. Bact.* **154**(3): 1431-1435, 1983.

535. FUJITA-YAMAGUCHI, Y., OISHI, K., SUZUKI, K. and IMAHORI, K. *Biochim. Biophys. Acta* **701**: 86-92, 1982.

536. TEODORESCU, M. *Biol. Cell* **51**: 251-258, 1984.

537. SUDAKEVITZ, D. and GILBOA-GARBER, N. *Microbios* **34**: 159-166, 1982.

538. SHARON, N. *Immunol. Today* **5**(5): 143-147, 1984.

539. JENSEN, C., STAHL SKOV, P., KOCH, C., PERMIN, H., BOG-HANSEN, T.C., LOWENSTEIN, H., HOIBY, N. and NORN, S. *Eur. J. Respir. Dis.* **64**(Suppl. 128): 391-393, 1983.

540. STAHL SKOV, P., JENSEN, C., NORN, S., BOG-HANSEN, T.C., LOWENSTEIN, H., KOCH, C., PERMIN, H. and HOIBY, N. In: Lectins - Biology, Biochemistry, Clinical Biochemistry. Vol. 3. Edited by T.C. Bog-Hansen and G.A. Spengler. Berlin: W. de Gruyter & Co., 1983. pp.27-36.

541. ALLEN, P.Z., CONNELLY, M.C. and APICELLA, M.A. *Can. J. Microbiol.* **26**: 468-474, 1980.

542. CURTIS, G.D.W. and SLACK, M.P.E. *Br. J. Vener. Dis.* **57**: 253-255, 1981.

543. CONNELLY, M.C., STEIN, D.C., YOUNG, F.E., MORSE, S.A. and ALLEN, P.Z. *J. Bact.* **148**(3): 796-803, 1981.

544. SCHALLA, W.O., WHITTINGTON, W.L., RICE, R.J. and LARSEN, S.A. *J. Clin. Microbiol.* **22**(3): 379-382, 1985.

545. SCHAEFER, R.L., KELLER, K.F. and DOYLE, R.J. *J. Clin. Microbiol.* **10**(5): 669-67, 1979.

546. YAJKO, D.M., CHU, A. and HADLEY, W.K. *J. Clin. Microbiol.* **19**(3): 380-382, 1984.

547. DOYLE, R.J., NEDJAT-HAIEM, F., KELLER, K.F. and FRASCH, C.E. *J. Clin. Microbiol.* **19**(3): 383-387, 1984.

548. COLE, H.B., EZZELL, J.W. Jr., KELLER, K.F. and DOYLE, R.J. *J. Clin. Microbiol.* **19**(1): 48-53, 1984.

549. DELUCCA, A.J. II. *Can. J. Microbiol.* 30:1100-1104, 1984.

550. CORBEL, M.J., COCKREM, D.S. and BREWER, R.A. *Vet Rec.* 113: 261-262, 1983.

551. DAVIDSON, S.K., KELLER, K.F. and DOYLE, R.J. *J. Clin. Microbiol.* 15(4): 547-553, 1982.

552. OTTENSOOSER, F., NAKAMIZO, Y., SATO, M., MIYAMOTO, Y. and TAKIZAWA, K. *Infect. Immun.* 9(5): 971-973, 1974.

553. MATTIASSON, B., LING, T.G.I., NILSSON, J. and DURHOLT, M. In: Lectins - Biology, Biochemistry, Clinical Biochemistry. Vol.2. Edited by T.C. Bog-Hansen. Berlin: W. de Gruyter & Co., 1982. pp. 573-581.

554. HART, D.A. *Amer. J. Clin. Nutr.* 33: 2416-2425, 1980.

555. PEDERSEN, I.R. and MORDHORST, C.H. In: Lectins - Biology, Biochemistry, Clinical Biochemistry. Vol.1. Edited by T.C. Bog-Hansen. Berlin: W. de Gruyter & Co., 1981. pp. 395-399.

556. POSTE, G. and REEVE, P. *Nature New Biol.* 237: 113-114, 1972.

557. ORAM, J.D., ELLWOOD, D.C., APPLEYARD, G. and STANLEY, J.L. *Nature New Biol.* 23:50-51, 1971.

558. BIRDWELL, C.R. and STRAUSS, J.H. *J. Virol.* 11(4): 502-507, 1973.

559. KLENK, H.-D., BECHT, H. and ROTT, R. *Ann. N.Y. Acad. Sci.* 234: 355-368, 1974.

560. REEVE, P., HEWLETT, G., WATKINS, H., ALEXANDER, D.J. and POSTE, G. *Nature* 249: 355-356, 1974.

561. LEHMAN, J.M. and SHEPPARD, J.R. *Virol.* 49: 339-341, 1972.

562. ISHIZAKI, R. and BOLOGNESI, D.P. *J. Virol.* 17(1): 132-139, 1976.

563. STEWART, M.L., SUMMERS, D.F., SOEIRO, R., FIELDS, B.N. and MAIZEL, J.V. Jr. *Proc. Nat. Acad. Sci. U.S.A.* 70(5): 1308-1312, 1973.

564. STITZ, L., REINACHER, M. and BECHT, H. *J. Gen. Virol.* 34: 523-530, 1977.

565. FINKELSTEIN, M.S. and MCWILLIAMS, M. *Virol.* 69: 570-586, 1976.

566. POSTE, G., REEVE, P., ALEXANDER, D.J. and TERRY, G. *J. Gen. Virol.* 17: 81-89, 1972.

567. POSTE, G., ALEXANDER, D.J., REEVE, P. and HEWLETT, G. *J. Gen. Virol.* 23: 255-270, 1974.

568. OKADA, Y. and KIM, J. *Virol.* 50: 507-515, 1972.

569. BENSON, L. Plant Classification. 2nd ed. Lexington, Mass.: D.C. Heath & Co., 1979.

570. HUTCHINSON, J. The Genera of Flowering Plants (Angiospermae) Dicotyledons. Vol.II. Oxford: Oxford at Clarendon Press, 1967.

571. CORNER, E.J.H. *Gard. Bull. Sing.* **19**: 187-252, 1962.

572. PURSEGLOVE, J. W. Tropical Crops; Dicotyledons 2. London: Longmans Green & Co. Ltd., 1968.

573. SMITH, A.C. Flora Vitiensis Nova A, New Flora of Fiji (Spermatocytes only) Vol. 2. Angiospermae + Dicotyledons, Families 44-116. Lawai, Kauai, Hawaii: Pacific Tropical Botanical Garden, 1981.

574. RENDLE, A.R. The Classification of Flowering Plants. Vol. II. Dicotyledons. Cambridge: Cambridge University Press, 1959.

575. BROWN, F.B.H. *B.P. Bishop Mus. Bull.* **130**. Honolulu, Hawaii: 1935. New York: Kraus Reprint Co., 1971.

576. FOSBERG, F.R. *Brittonia* **12**: 101-113, 1960.

577. JARRETT, F.M. *J. Arnold Arb.* **40**: 1-37, 113-155, 298-368, 1959.

578. JARRETT, F.M. *J. Arnold Arb.* **41**: 73-140, 320-340, 1960.

## II. SCREENING OF CRUDE SEED EXTRACTS

### A. Introduction

Since lectins have many applications and are potentially useful in a variety of fields, attempts were made to search for lectins from new plant sources. For this purpose, 23 species representing 10 botanical families including one gymnosperm (Table II-1, page 65) were screened using hemagglutination and Ouchterlony immunodiffusion assays.

The immunochemical analysis of lectins has proven valuable as a tool for defining phylogenetic relationships and chemotaxonomical classifications. Concanavalin A and lentil lectins are thought to have a common ancestor[1] and it appears from the sequence homologies determined to date that the genes coding for leguminous lectins in general share a common ancestry[2-4]. Ouchterlony immunodiffusion assays were conducted to determine if this type of relationship also extends to Moraceae agglutinins.

In addition to the above immunochemical/serological procedures, bacterial agglutinability of Moraceae extracts were carried out to identify potential applications, such as a means to quickly distinguish bacterial species.

### B. Methods

1) Materials

Plant seeds were obtained from the various sources listed in the Appendix. Antisera directed against eight lectins (see page 65) were purchased from Sigma Chemical Co. Sugars used in the immunodiffusion medium were bought from Aldrich Chemical Co., except for lactose (Pfanstiehl Labs Inc.).

2) Preparation of crude seed extracts

One gram of seeds was ground using a mortar and pestle, mixed with 10 ml of 10 mM phosphate buffered saline (PBS) and tumbled overnight at 4°C. After centrifuging for 15 min.

at 320 *x g* in a Dynac table top centrifuge, the supernatant was collected and used as such or stored at -20°C.

3) Screening of crude seed extracts

Hemagglutination assays were conducted in plastic microtiter plates with samples added in 100 ul doubling dilutions and 75 ul of a 1.5% suspension of human erythrocytes in PBS. Fresh red blood cells provided by the Canadian Red Cross Blood Transfusion Service washed four times with PBS were used for this assay. *Dolichos biflorus*, an $A_1$ specific lectin, was used as an internal control. Ouchterlony immunodiffusion[5] was carried out in plastic Petri plates filled with 15 ml of 1.5% agar containing 0.05% sodium azide and 10 mg/ml concentrations of lactose, D-glucose, D-galactose, L-fucose, N-acetyl-α-D-glucosamine, D-mannose and D-maltose monohydrate. The sugars were added to eliminate any spurious precipitin lines due to lectin-glycoprotein interactions. Wells were filled with 20 ul of antisera or extract and incubated overnight in a humid chamber. Precipitin lines observed were washed for two days with 0.9% NaCl to remove non-involved proteins, dried using Whatman 1 filter paper, then stained for 18 min. with amido black and destained with 2% acetic acid.

4) Bacterial culturing and agglutination

The following bacteria were cultured on nutrient agar at 37°C: *Escherichia coli* 10418, *Proteus vulgaris* 13315, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* 13883, *Salmonella oranienburg* and *Staphylococcus aureus* 6538P (FDA 209P). *Bacillus cereus* and *Micrococcus luteus* 10240 were grown on the same media at 30°C, while *Serratia marcescens* was incubated at 26°C. *Staphylococcus aureus* (strains N2, a laboratory strain which is a producer of penicillinase, and 25923) was grown on blood agar plates at 37°C. *Salmonella typhimurium* 13311, *E. coli* (B strain 11303 and ESS, a mutant strain very sensitive to B-lactam antibiotics) were cultured on trypticase soy agar at 37°C. *Micrococcus luteus* 9341 and *Comamonas terrigena* 8461 were grown on the same media at 30°C.

A loop of bacteria was stirred in 100 µl of water on a glass slide, 100 µl extract added, rocked for a few minutes, and observed for agglutination reactions.

*Chlamydia trachomatis* serovar $L_2$ was harvested from infected monolayers cultivated in HeLa cells and elementary bodies were purified by layering over a discontinuous Renografin (Squibb) gradient and centrifugation at 43,000 *x g* for 60 min. at 4°C. Elementary bodies were collected from the 44-52% interface and diluted with three volumes of N-tris(hydroxymethyl)methyl 2-aminoethanesulfonic acid (Sigma Chemical Co.) buffer (TES). This preparation was centrifuged for 30 min. at 30,000 *x g* and 4°C. The purified material was resuspended in 10 mM TES in 0.85% NaCl and stored at -70°C. Agglutination tests were performed on glass slides and viewed under a microscope.

**C. Results and Discussion**

The extracts prepared from the two *Artocarpus* species and *Ficus deltoidea* displayed a high hemagglutination titer for A, B, O and AB human erythrocytes. *Mangifera indica* exhibited a slight preference for blood group O, despite its ability to agglutinate the other blood types as well. Moderate hemagglutination was observed with *F. vogelii* and *A. squamosa*. Weak agglutination activity was detected in *A. cherimolia*, *H. japonicus*, *B. papyrifera*, *D. contrajerva*, *P. mungo*, *P. aconitifolius*, *L. chinensis* and the two *Brassica* species. No activity was seen in the four Pinaceae species, *D. virginiana*, *P. radiatus*, *M. pumila* and *C. sativa*. One feature of the current investigation was to examine readily available Canadian seeds and taxa, including gymnosperms. These species produced low titers (*Brassica*) or failed to agglutinate human red blood cells (Pinaceae species) (Table II-2, page 66). A sample of *A. integrifolia* from Bangalore, India produced an exceptionally high titer (1/25,600) compared to a sample from Madras, India (1/64). This may be the result of using seeds which have been ground to a fine powder, as supplied by the Bangalore source. Grinding intact seeds from Madras using a mortar and pestle produced much lower titers suggesting this approach is not nearly as efficient or that large strain or variety differences may exist in this species.

The absence of agglutination or low titers in these results may not give an entirely accurate picture of lectin content, since no provision was made to remove oils, fats and other materials which could have been responsible for low lectin yields. The two *Brassica* species are particularly rich in oils and utilized commercially for canola oil. In addition, untreated blood was used in the assays and a minority of lectins are known to require pretreatment with neuraminidase, trypsin or papain for their agglutination activity. A third factor involves regional differences and variations in plants. It is also possible the lectin could be localized in a part of the plant other than the seeds. Agglutination could be prevented by the existence of glycoprotein-lectin complexes covering the active site of the lectin as well. Thus, while Hardman *et al*[8] also failed to detect a lectin in *C. sativa*, agglutination material may be present

as suggested by Tumosa[7-8]. As a technique, hemagglutination is simple and rapid but only semiquantitative at best. The results are subject to varied interpretation and provide few details as to specificity.

Antisera directed against *Maclura pomifera*, *Arachis hypogaea*, *Triticum vulgaris*, *Dolichos biflorus*, *Canavalia ensiformis*, *Limulus polyphemus*, *Tetragonolobus purpureas* and *Pisum sativum* agglutinins were used in the Ouchterlony immunodiffusion assays. The *F. deltoidea* extract showed a precipitin line with all of the above antisera. However, these precipitin reactions were eliminated when the crude FDA extract was mixed with normal rabbit sera, indicating the formation of a non-specific precipitin line with serum (see also chapter IV). Both *Artocarpus* extracts showed cross-reactivity with antisera directed against *M. pomifera* (see also chapter III). The remaining species did not produce precipitin lines with any of the eight antisera used. The spurs obtained with *Artocarpus* indicate a reaction of partial identity. Spur formation gives an indication of whether two proteins are antigenically similar or distinct. A single spur results when two different antigens share some common determinant(s)[9]. Three types of precipitation patterns are recognized (Figure II-1, page 68); A pattern of identity is represented by one continuous line indicating both antigens possess identical determinants with respect to the antiserum used. In a reaction of nonidentity, the lines form independently of each other without significant interaction meaning the antiserum does not contain antibodies directed against any determinant groups common to both antigens. In partial identity, a spur is generated by determinants found in one antigen but not the other. It is a case of one antigen reacting more fully with the antiserum than the second antigen. Thus the cross-reacting *Artocarpus* lectins are immunologically related, but not identical to the lectin from *Maclura pomifera*. Different proteins can be functionally similar while totally unrelated antigenically. But antigenically similar proteins possess a strong probability of both a functional and evolutionary relationship[10]. Cross-reactivity has been found among some lectins within the Solanaceae[11-12] and Leguminosae[10,13-14]. The present study suggests lectins within the Moraceae family may also be related. Cross-reactivity does seem to occur between

families as indicated in both literature and current investigations.

Precipitin lines were generated between some pairs of lectins in the outer wells of the agar plates, including *M. indica* and *P. mungo*, and *M. indica* and *A. squamosa*. This observation of reactivity indicating an apparent relationship between a member of Anacardiaceae and Leguminosae, and Anacardiaceae and Annonaceae, was further investigated to eliminate the possibility of lectin-lectin interaction (where at least one should be a glycoprotein). No precipitin lines were obtained between the same pairs of seed extracts when immunodiffusion plates containing lactose, D-glucose, D-galactose, L-fucose, N-acetyl-α-D-glucosamine, D-mannose and D-maltose monohydrate were used. Analogous to these spurious lines, false reactivities could also be generated due to the presence of glycoproteins in serum. Such false positives were also eliminated by sugars in the media, leaving only true antigen/antibody reactions.

Preliminary experiments showed samples of FDA agglutinated trypticase soy broth, nutrient broth and trypticase, so bacteria were cultured on agar rather than in broth. Slight agglutination by the *F. deltoidea* extract was observed with *Staphylococcus aureus* and *Comamonas terrigena* and fair agglutination was seen using *Bacillus cereus*, *S. aureus* N2 and *Salmonella typhimurium*. *Chlamydia trachomatis* produced a strong agglutination. The other species and strains tested failed to agglutinate with the crude FDA. *Broussonetia papyrifera*, the only other Moraceae extract to interact with bacteria, slightly agglutinated *B. cereus* and *C. trachomatis* (Table II-3, page 67). *Chlamydia* causes the most prevalent sexually transmitted disease in Canada and is difficult to diagnose because of its asymptomatic nature. It can result in scarring of the fallopian tubes, infertility and pelvic inflammatory disease in women and epididymitis and prostitis in men. In addition, one-third of children born to infected mothers develop conjunctivitis, an eye infection. Due to its agglutination of this bacterium, FDA may be a clinically important lectin.

Table II-1. Species examined in the current studies.

| CLASS | FAMILY | SPECIES | COMMON NAME |
|---|---|---|---|
| Gymnospermae | Pinaceae | *Larix sibirica* Ledeb | Siberian larch |
| | | *Picea glauca* (Moench) Voss | white spruce |
| | | *Pinus contorta* Loudon var. latifolia Engelm | lodgepole pine |
| | | *Pseudotsuga menziesii* (mirb.) Franco | Douglas fir |
| Angiospermae | Ebenaceae | *Diospyros virginiana* Linn. | persimmon |
| | Sapindaceae | *Litchi chinensis* Sonner. (*Nephelium litchi* Linn.) | lychee |
| | Leguminosae | *Phaseolus mungo* Linn. | black manh, black bean |
| | | *Phaseolus aconitifolius* Jacq. | moth |
| | | *Phaseolus radiatus* (L.) Wilzeck | red chori |
| | Rosaceae | *Malus pumila* Mill. cultivar McIntosh | McIntosh apple |
| | Cruciferae | *Brassica campestris* Linn. | Polish rapeseed |
| | | *Brassica napus* Linn. | Argentina rapeseed |
| | Moraceae | *Ficus vogelii* Miq. | African tropical fig |
| | | *Ficus deltoidea* Jack. (*F. diversifolia* Blume) | mistletoe fig |
| | | *Dorstenia contrajerva* Linn. | American tropical fig |
| | | *Artocarpus integrifolia* Linn. | jackfruit |
| | | *Artocarpus altilis* (Parkinson) Fosberg | breadfruit |
| | | *Broussonetia papyrifera* Vent. | paper mulberry |
| | Cannabinaceae | *Cannabis sativa* Linn. | hemp |
| | | *Humulus japonicus* Sieb. & Zucc. | Japanese hop |
| | Annonaceae | *Annona squamosa* L. | sitaphal, custard apple |
| | | *Annona cherimolia* Mill. | cherimoya |
| | Anacardiaceae | *Mangifera indica* Linn. | mango |

Table II-2. Hemagglutination and Ouchterlony immunodiffusion studies on extracts from a variety of plant seeds.[1]

| SPECIES | SOURCE | HEMAGGLUTINATION TITER[2] | | | | OUCHTERLONY ASSAY |
|---|---|---|---|---|---|---|
| | | Human Blood Groups | | | | *Maclura pomifera* antisera |
| | | A | B | O | AB | |
| *Larix sibirica* | Local | 0 | 0 | 0 | 0 | - |
| *Picea glauca* | Local | 0 | 0 | 0 | 0 | - |
| *Pinus contorta* | Local | 0 | 0 | 0 | 0 | - |
| *Pseudotsuga menziesii* | Local | 0 | 0 | 0 | 0 | - |
| *Diospyros virginiana* | Local | 0 | 0 | 0 | 0 | - |
| *Litchi chinensis* | Local | 4 | 4 | 4 | 4 | - |
| *Phaseolus mungo* | Local | 64 | 16 | 64 | 64 | - |
| *Phaseolus aconitifolius* | Local | 1 | 1 | 0 | 0 | - |
| *Phaseolus radiatus* | Local | 0 | 0 | 0 | 0 | - |
| *Malus pumila* | Local | 0 | 0 | 0 | 0 | - |
| *Brassica campestris* | Local | 1 | 1 | 1 | 1 | - |
| *Brassica napus* | Local | 1 | 1 | 1 | 1 | - |
| *Ficus vogelii* | Redwood City, Ca. | 128 | 128 | 128 | 128 | - |
| *Ficus deltoidea* | Local | 6400 | 6400 | 6400 | 6400 | +[3] |
| *Dorstenia contrajerva* | Local | 32 | 16 | 16 | 16 | - |
| *Artocarpus integrifolia*[4] | Madras, India | 64 | 64 | 64 | 64 | + |
| *Artocarpus altilis* | Local | 2048 | 1024 | 1024 | 1024 | + |
| *Broussonetia papyrifera* | Sandwich, Mass | 2 | 2 | 2 | 2 | - |
| *Cannabis sativa* | Ottawa, Ont | 0 | 0 | 0 | 0 | - |
| *Humulus japonicus* | Redwood City, Ca | 8 | 8 | 8 | 4 | - |
| *Annona squamosa* | Local | 64 | 128 | 128 | 128 | - |
| *Annona cherimolia* | Local | 40 | 40 | 40 | 40 | - |
| *Mangifera indica* | Local | 1024 | 2048 | 4096 | 2048 | - |

[1] *Dolichos biflorus* agglutinin, an $A_1$ specific lectin, was used as a reference in hemagglutination studies.
[2] Titer refers to the reciprocal of the last doubling dilution showing agglutination.
[3] Positive results were also obtained with normal sera and therefore represent non-specific precipitin reactions.
[4] Powdered seeds from Bangalore, India source showed a surprisingly high titer of 25,600.

Table II-3. Bacterial agglutination tests with extracts from Moraceae species.

| BACTERIUM | DESCRIPTION | F. DELTOIDEA[1] | B. PAPYRIFERA[1] |
|---|---|---|---|
| *Staphylococcus aureus* | Gram + cocci | + | - |
| *Staphylococcus aureus N2* | | ++ | - |
| *Micrococcus luteus* | Gram + cocci | - | - |
| *Bacillus cereus* | Gram + endospore-forming rods | ++ | + |
| *Chlamydia trachomatis* | Gram - spheroidal | +++ | + |
| *Klebsiella pneumoniae* | Gram - facultatively anaerobic rods | - | - |
| *Salmonella oranienburg* | Gram - facultatively anaerobic rods | - | - |
| *Salmonella typhimurium* | | ++ | - |
| *Serratia marcescens* | Gram - facultatively anaerobic rods | - | - |
| *Pseudomonas aeruginosa* | Gram - aerobic rods | - | - |
| *Proteus vulgaris* | Gram - facultatively anaerobic rods | - | - |
| *Escherichia coli* | Gram - facultatively anaerobic rods | - | - |
| *Escherichia coli B* | | - | - |
| *Escherichia coli ESS* | | - | - |
| *Aeromonas terrigena* | Gram - facultatively anaerobic curved rods | + | - |

Note: Extracts prepared from *F. vogelii*, *D. contrajerva*, *A. integrifolia* and *A. altilis* did not agglutinate any of the bacterial species tested.
[1] Agglutination was assessed visually as weak (+), medium (++), strong (+++) or absent (-).

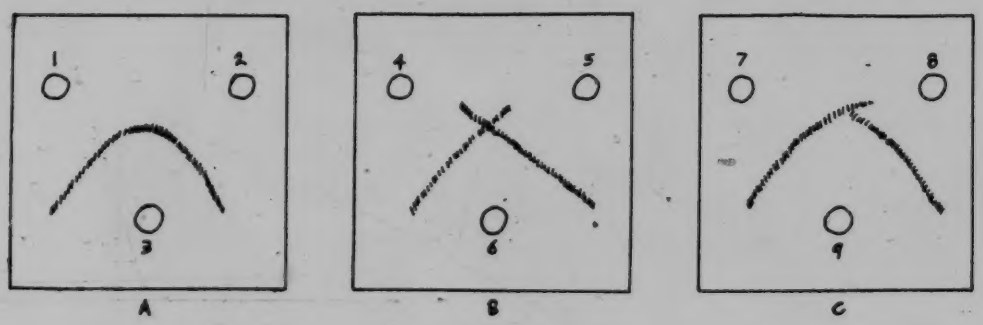


Figure II-1. Illustrations of Ouchterlony immunodiffusion precipitin reactions in agar. In the reaction of identity (A), wells 1 and 2 contain the same antigen with the antisera in well 3. A reaction of nonindentity (B) was obtained by placing different antigens in wells 4 and 5 and antisera to both in well 6. Partial identity (C) was achieved by using an antigen and its antiserum in wells 7 and 9, respectively, and a crossreacting antigen in well 8. (Adapted from Davis, B.D. *et al*, 1980).

## D. References


1. FORIERS, A., DE NEVE, R., KANAREK, L. and STROSBERG, A.D. *Proc. Natl. Acad. Sci.* **75**: 1136-1139, 1978.

2. FORIERS, A., WUILMART, C., SHARON, N. and STROSBERG, A.D. *Biochem. Biophys. Res. Comm.* **75**(4): 980-986, 1977.

3. STROSBERG, A.D., LAUWEREYS, M. and FORIERS, A. *Prog. Clin. Biol. Res* **138**: 7-20, 1983.

4. FORIERS, A., DE NEVE, R. and STROSBERG, A.D. *Physiol. Veg.* **17**(3): 597-606, 1979.

5. GOLDSTEIN, I.J. and SO, L.L. *Arch. Biochem. Biophys.* **111**: 407-414, 1965.

6. HARDMAN, J.T., BECK, M.L. and OWENSBY, C.E. *Transfusion* **23**(6): 519-522, 1983.

7. TUMOSA, C.S. *Transfusion* **24**(5): 414, 1984.

8. TUMOSA, C.S. *Experientia* **40**(7): 718-719, 1984

9. OUCHTERLONY, O. and NILSSON, L. In: Handbook of Experimental Immunology. Edited by D.M. Weir. Oxford: Blackwell Scientific Publications, 1973. pp. 19.17-19.20.

10. SHANNON, L.E. In: Lectins - Biology, Biochemistry, Clinical Biochemistry. Vol. 3. Edited by T.C. Bog-Hansen and G.A. Spengler. Berlin: W. de Gruyter & Co., 1983. pp. 573-581.

11. KILPATRICK, D.C. *Prog. Clin. Biol. Res.* **138**: 63-70; 1983.

12. NACHBAR, M.S. and OPPENHEIM, J.D. *Methods Enzymol.* **83**: 363-368, 1982.

13. SHANNON, L.M. *Prog. Clin. Biol. Res.* **138**: 47-61, 1983.

14. HOWARD, J., KINDINGER, J. and SHANNON, L. M. *Arch. Biochem. Biophys.* **192**(2): 457-465, 1979.

15. DAVIS, B.D., DULBECCO, R., EISEN, H.N. and GINSBERG, H.S. Microbiology. 3rd ed. Hagerstown, Md.: Harper & Row, 1980.

## III. STUDIES ON A LECTIN FROM *ARTOCARPUS ALTILIS* SEEDS *

### A. Introduction

*Artocarpus altilis*[1] (*A. communis*, *A. incisa*) or breadfruit is cultivated throughout the tropics but is indigenous to New Guinea, the Moluccas, Micronesia and western Melanesia regions of the South Pacific[2,3]. It was introduced to the West Indies by Capt. William Bligh in 1793[4]. The breadfruit has remained a tropical/subtropical plant since it requires hot, moist climates with temperatures of 25-30°C, 150-250 cm annual rainfall and well-drained soils[5].

The tree ranges from 40 to 60 feet (12-18 metres) in height, with many large, glossy green leaves arranged spirally on the branches. The plant is monoecious with male and female flowers in separate groups on the same tree. Ripe fruits measure 4-8 inches (10-20 cm) in diameter and are characterized by a thick, green to brownish-yellow rind enclosing a pale white, starchy, fibrous pulp[6,7] (Plates III-1,2; Figure III-1, pages 92 and 89).

The considerable amounts of starch and sugars present make breadfruit a staple food and it can be cooked and prepared in a number of ways[8]. In addition to being a good source of vitamin B, it is also a fair source of vitamins A and C[9]. Its potential for commercial processing has also been studied[10].While breadfruit is mainly a food staple, the plant has been utilized in a variety of ways. The wood has been used to make canoes and furniture, the latex for caulking material and glue, the fibrous inner bark transformed into cloth and dried leaves used for mats or polishing bowls[11,12].

Detailed scientific studies of the breadfruit are lacking. Phytochemical investigations have detected sterols and triterpenes in the fruit and bark[13-15]. One other study[16] analyzed ground breadfruit seeds and found dried samples contained 3.4% ash, 12.8% fat, 16.0% soluble carbohydrates, 20.0% total proteins and 3.9% crude fiber. The content of globular proteins was twice that of non-globular proteins.

---

*A version of this chapter has been accepted for publication in Biochemical Archives, [illegible]

As alluded to in the previous chapter, cross-reactivity was observed between a breadfruit extract and antisera directed against *M. pomifera*. As a result it was decided to purify the agglutinating material. This chapter deals with the isolation and characterization of a lectin from breadfruit seeds.

**B. Material and Methods**

1) Materials

Breadfruit imported from Fiji was obtained from a local store. The Synsorb™, carbohydrate-coated latex beads and synthetic carbohydrate haptens were provided by Chembiomed Ltd. (Edmonton). All chemicals and reagents used were of analytical grade.

2) Purification of breadfruit agglutinin

Preliminary studies on the distribution indicated the lectin was primarily localized in the seeds (Table III-1, page 82). The average breadfruit yielded 10 seeds weighing a total of about 40 g (fresh).

In a typical purification, the seed coats were removed and 78 g of breadfruit seeds along with 780 ml of PBS (1:10 W/V) ground in a blender for 1 min. The mixture was sonicated on ice with three 30 sec bursts (0.2 relative output, Fisher Sonic Dismembrator Model 300) and stirred overnight at 4°C. The crude breadfruit extract was centrifuged for 20 min. at 2100 *x g* and 4°C. The supernatant was filtered through glass wool. 1M $MnCl_2$ (5.8 ml) was added dropwise with stirring to remove nucleoproteins from the filtrate. The mixture was centrifuged as before and the pellet discarded. The supernatant was brought to 30% saturation of $(NH_4)_2SO_4$ and stirred on ice for 30 min.[17] The extract was centrifuged and the pellet discarded. The supernatant was subsequently brought to 75% saturation of $(NH_4)_2SO_4$ and stirred on ice for 30 min. After centrifugation the pellet was resuspended in 150 ml PBS and dialyzed for 2 days at 4°C against 2L of PBS with two changes. Following a preliminary characterization using sugar-bound latexes (see later section), the dialyzed extract was added to T-Synsorb™ (immobilized β-D-gal(1→3)αgalNAc with an incorporation of 1.89 μm of T

hapten/g of Synsorb™) and tumbled for 4 h at room temperature. The Synsorb™ was then washed with 500 ml of PBS and poured into a column. Washings were continued until the absorption at 280 nm registered a baseline. Elution was carried out using a pH gradient prepared from 10 mM Tris-HCl - 10% sucrose (added as a protein stabilizer) - 0.9% NaCl - 0.1% $NaN_3$ (pH 7) and 10 mM acetic acid - 10% sucrose - 0.9% NaCl (pH 2), followed by an additional 100 ml of the same solution. Ten ml fractions were collected and pooled aliquots containing the eluted peak neutralized with 0.5M $Na_2HPO_4$. This material was concentrated to 25 ml in an Amicon Ultrafitration cell model 402 with a PM-10 membrane under 25 psi nitrogen (Table III-2, page 83). The 78 g of breadfruit seeds yielded approximately 15 mg of purified lectin (by weight after lyophilization). All of the fractions from the different purification steps were tested for hemagglutination as described in the previous chapter. The units of activity were determined by multiplying the titratable endpoint of a given fraction using human red blood cells and doubling dilution of the fraction by the volume. Specific activity represents the units of activity /g protein.

3) Characterization of breadfruit agglutinin

a. Latex agglutination assay

The partially purified (after salt precipitation and dialysis) breadfruit seed extract was tested for agglutination against a series of carbohydrate-bound latexes (developed by Chembiomed Ltd. and described in reference 18) in order to quickly identify the sugar specificity. Based on the results obtained (Table III-3, page 84), a 1:10 dilution of BFA extract in 3% BSA was adsorbed overnight on 50 mg of three different Synsorbs™ ($\beta$gal(1→3)$\alpha$galNAc, $\alpha$gal, $\beta$gal(1→4)$\beta$glcNAc) and a control. After centrifugation in an Eppendorf microfuge for 4 min., 100 ul of the supernatants were diluted serially in PBS. One hundred ul of 2% red blood cells were added and the tubes allowed to sit at room temperature for 1 h before checking for agglutination. Weak agglutinating activity would be indicative of BFA binding to the specific Synsorb™. The results suggested the T-Synsorb™ ($\beta$gal(1→3)-$\alpha$galNAc) was the most suitable adsorbent to use in an affinity chromatography column.

b. Radioligand inhibition studies

i) Preparation of RIA tubes

A solution of purified BFA containing 50 ug protein/ml in dilution buffer (50mM $NaH_2PO_4/Na_2HPO_4$, 5 mM $MgCl_2$, 15mM $NaN_3$ pH 7.5) was prepared and 100 ul used to coat RIA tubes[19,20]. After an overnight incubation at room temperature, the coating solution was aspirated and 200 ul of 5% BSA in PBS added to prevent non-specific binding. Following a 4 h incubation at room temperature the BSA solution was removed and replaced by 1.9 ml of PBS for 10 min. The RIA tubes were aspirated three times to thoroughly remove the PBS and air dried for 1 h at room temperature. The coated tubes were stored at 4°C in a sealed plastic bag..

ii) $^{125}I$-labeling of T-BSA

One mg of iodogen (1,3,4,6-tetrachloro-3$\alpha$, 6$\alpha$-diphenyl-glycouril) was dissolved in 100 ml of chloroform. One hundred ul of this solution was dispensed into a tube and dried under a gentle nitrogen stream until ready to use. A 1 mg/ml solution of T-hapten-BSA ($\beta$-D-gal(1→3)$\alpha$galNAc-BSA) (Chembiomed Ltd.) to be labeled was prepared in iodination buffer (0.5M $NaH_2PO_4/Na_2HPO_4$ pH 7.1). Twenty ul of iodination buffer was placed in another tube, 10 ul of $Na^{125}I$ (4000 MBq/ml in 0.1M NaOH) added and mixed thoroughly. Following the addition and mixing of 10 ul of the T-BSA solution, the contents were transferred to the iodogen-plated tube, stoppered and incubated for 45 min. with occasional gentle agitation. The sample was removed from the iodogen tube to a fresh tube and 20 ul of 1M NaI and 50 ul of 1% BSA/PBS added. This was incubated for 40 min. to allow non-radioactive $I^-$ to exchange with any non-specifically bound $^{125}I^-$.

iii) Gel filtration chromatography of $^{125}$I-labeled T-BSA

Gel filtration chromatography is required to separate the labeled T-BSA from radioactivity which did not bind (usually in form of $I^-$). A 1 by 10 cm Bio-Gel P6DG column was washed with 1 ml of 1% BSA/PBS followed by 20 ml of PBS. Column chromatography was initiated by washing the labeled sample in with small applications of PBS, connected to a PBS reservoir. At approximately 1.5 ml prior to the void volume, 8-drop fractions were collected in tubes previously exposed to a Zerostat™ gun to remove static. After collecting the last fraction the column was washed with 20 ml PBS. The five or six fractions containing the bulk of the radioactivity were pooled and a 1/100 dilution of radioactive stock prepared. A working solution of about 9.65 uCi (250,000 cpm)/ 50 ul was derived from the radioactive stock and 1% BSA/PBS added to bring the volume to 40 ml.

iv) Inhibition of $^{125}$I-T-BSA binding

Two mg/ml concentration in PBS of 13 free haptens (Table III-4, page 85) were screened in triplicate for inhibition of $^{125}$I-T-BSA binding to BFA coated RIA tubes. Three coated (as described above) and three uncoated tubes contained 100 ul 1% BSA in PBS, while the remaining coated tubes received 100 ul of hapten. Fifty ul of $^{125}$I-labeled T-BSA solution was added to all the tubes. The following day, tubes were aspirated, washed three times with PBS and measured for radioactivity in a Beckman Gamma 400 spectrometer. To determine the 50% inhibition value of the T-hapten, serial dilutions of hapten from 2-500 ug/ml in 1% BSA in PBS were prepared and the procedure described above repeated.

c. Gel filtration

Five g of Bio-Gel P150 (100-200 mesh) were hydrated in PBS for 4 hr, then degassed under vacuum and poured into a column. Bed height measured 48 cm and column diameter, 1.5 cm. Uniform packing of the column and void volume were checked using 2 ml of blue dextran (10 mg/ml). A flow rate of 12 ml/hr was established (LKB Bromma 2132 Microperpex peristaltic pump) and absorbance monitored at 280 nm. A 1 ml sample

containing 1 mg of each of the following molecular weight markers (Sigma Chemical Co.) were used to calibrate the column: Cytochrome C from horse heart (12,400), trypsinogen from bovine pancreas (24,000), ovalbumin (45,000), albumin from bovine serum (66,000), alcohol dehydrogenase from yeast (150,000) and $\beta$-amylase from sweet potato (200,000). Gel filtration[21] was also conducted using a Pharmacia FPLC system with a Pharmacia HR 10/30 Superose 12™ high performance gel filtration column (chapter IV). After calibration, 1 ml of purified BFA was chromatographed and its Ve determined.

d. SDS polyacrylamide gel electrophoresis

Electrophoresis was performed in 20% (w/v) polyacrylamide slab gels of 1.5 mm thickness, in the presence of 0.1% SDS, employing the discontinuous buffer system of Laemmli[22]. The gels were run at 30 mAmps per slab using a Bio-Rad Protean 16CM unit. Reduced gels were obtained with 10 mM dithiothreitol (DTT). Gels were stained on a shaking bath with 0.15% (w/v) Coomassie Brilliant Blue R-250 in 50% ethanol/10% acetic acid, and destained in 20% ethanol/10% acetic acid.

e. Isoelectric focusing in polyacrylamide gels

Isoelectric focusing gels were cast in glass tubes of 2.5 mm internal diameter and 15 cm length. The gel composition was: 4% acrylamide, 0.24% N,N'-methylene bis acrylamide, 9M urea, 0.66% NP40 and 2% pH 3.5-10 ampholines (LKB Chemical). Samples were dissolved in freshly prepared buffer containing 9M urea and 1 mM DTT, centrifuged to remove insoluble material and then loaded onto gels and subjected to nonequilibrium pH gradient electrophoresis[23]. The extracts were run for 16 h at 250 volts, making a total of 4000 Vhrs. Gels were stained with Coomassie Brilliant Blue R-250, after pretreatment with 15% trichloroacetic acid (TCA) (three changes in three h) and 20% ethanol/10% acetic acid (four changes in two h), to remove ampholines. Approximate isoelectric points were determined by sectioning the gels, immediately after electrophoresis, into 1 cm segments and eluting the ampholines into 1 ml of degassed water over a period of 10 min. and measuring the pH. The

gradient obtained for the major central region of the gel was found to be linear with a ΔpH value of 0.04/mm gel.

f. Two-dimensional gel electrophoresis

Two-dimensional gel electrophoresis was performed by running an isoelectric focusing gel in the first dimension followed by SDS PAGE in the second dimension[24-26].

g. Amino acid analysis

Timed acid hydrolysis of 24, 48, 72, and 96 h was conducted on 200 ug samples of purified BFA which had been mixed with 1 ml 6N HCl in sealed glass tubes, evacuated and hydrolyzed at 110°C. After removing the HCl by evaporating to dryness and dissolving the residue in sodium citrate buffer pH 2.2, 0.5 ml aliquots were analyzed on a Durrum amino acid analyzer[27].

h. Carbohydrate determination

The phenol-sulfuric acid method[28] was employed to determine the sugar content of the purified lectin. Galactose standards containing 0, 10, 25, 50, 100 or 200 ug/ml $dH_2O$ were prepared. One ml of 5% phenol was added, followed by 5 ml of conc. $H_2SO_4$ with shaking. Colour development was read at 490 nm in a Bausch and Lomb Spectronic 2000 spectrophotometer after 30 min. One aliquot of BFA was analyzed without further alteration. A second sample was tested after being passed through a Bio-Gel P150 column equilibrated and eluted with 0.1M $NH_4OH$ to dissociate complexes, then neutralized with 0.1M HCl[29].

i. Divalent ion dependency

Two ml of purified lectin was dialyzed against 2L of 10 mM Tris-HCl pH7 containing 50 mM NaCl and 100 mM diNa EDTA dihydrate, while a second aliquot of BFA was dialyzed against 2L of PBS for two days. The sample which had been dialyzing in EDTA was then dialyzed against 1L of PBS for two days. Hemagglutination was conducted on both

dialyzed samples[30].

j. Densitometry

BFA protein bands on the SDS PAGE gels were analyzed using a Joyce Loebl Chromoscan 3 densitometer, scanning from the bottom of the gel to the top.

k. Preparation of antisera in rabbits

One ml of purified BFA (0.6 mg) was mixed with 1 ml complete Freunds' adjuvant and vortexed thoroughly to form an emulsion. One-half ml was injected intramuscularly into four sites of female New Zealand white rabbits weighing about 4 kg each. The animals received a booster injection of BFA after five weeks and were bled from the marginal vein of the ear one week later. The blood was placed in a water bath at 37°C for 30-35 min., then refrigerated at 4°C overnight. The serum was collected and centrifuged for 15 min. at 450 *x g* in a Dynac centrifuge. The supernatant was frozen at -20°C following the addition of 0.1M sodium azide.

## C. Results and Discussion

The extracts prepared from the two *Artocarpus* species displayed a high hemagglutination titer for A, B, O and AB human erythrocytes (chapter II). Both showed cross-reactivity with antisera directed against *Maclura pomifera* agglutinin, as evidenced by the presence of spurs (Plates III-3 i. and ii. and 4, page 93). The jackfruit precipitin line (Plate III-4, page 94) was clearer in other cases, but gave the same appearance. The spurs generated indicate a reaction of partial identity, suggesting lectins within the Moraceae family are immunochemically related.

The breadfruit agglutinin (BFA) was purified to homogeneity using an affinity chromatography procedure employing T-Synsorb™ in view of its apparent binding specificity towards the disaccharide based on latex agglutinations and preliminary screening. $MnCl_2$ treatment to remove nucleoproteins increased the specific activity marginally, compared to the 67-fold enrichment achieved by $(NH_4)_2SO_4$ salt fractionation (Table III-2, page 83). The affinity chromatography step produced a 147-fold enrichment (determined by hemagglutination assays) resulting in a homogeneous product. The apparent increases in yields observed in the $(NH_4)_2SO_4$ fractionation steps is perhaps indicative of the removal of a soluble inhibitor. It is relevant to draw attention to the fact that substantial amounts of sugars are co-purified along with the lectin (see Table III-5, page 86). It is likely that the high salt content could dissociate some of the saccharides bound to the lectin active site and thus enhance total activity. Homogeneity of the protein was shown by the following biochemical techniques: A single peak on gel filtration (Figure III-2, page 90) and a single precipitin line in Ouchterlony immunodiffusion analysis (Plates III-3 i. and ii., page 93). Further support to the purity of the lectin preparation is indicated by SDS polyacrylamide gel electrophoresis (Plate III-5 lanes 2 and 3, page 95) wherein the seed proteins are essentially absent in the purified sample. The two-dimensional electrophorectic gels also indicate the extent of purification (Plate III-7, page 97).

No difference in the titer was observed following dialysis of BFA against 100 mM EDTA. This suggests divalent ions are not required for activity or are too tightly bound to be removed by the method employed. Galbraith and Goldstein[30] were able to completely inhibit the precipitation reaction caused by lima bean lectin using 0.1 mM EDTA, a 1000-fold lower concentration.

The carbohydrate determination indicated that BFA is a glycoprotein containing 4.3% carbohydrate (Table III-5, page 86). However, a sample not run through the gel filtration column with 0.1M $NH_4OH$ apparently showed 210% carbohydrate implying that other glycans are copurified with the lectin. Thus, the use of $NH_4OH$ to dissociate complexes appears to be well founded and could have influenced the final yield of lectin from the crude extract. By comparison, the jackfruit lectin is reported to have 3% carbohydrate[31].

Gel filtration of BFA through a column of Bio-Gel P150 produced a single symmetrical peak around 44,500 in molecular weight (Figure III-2, page 90). This value is subject to some error in view of the fact BFA is a glycoprotein[32]. The figure estimated may be high, owing to the copurification of other glycans. Two dissimilar components of 19,000 and 22,000 daltons were seen on the SDS reduced electrophoretic gel (Plate III-5, page 95). The non-reduced gel gave a similar pattern. The pattern was not altered in the absence of reducing agents such as DTT which suggests that subunit interactions are not through a disulfide bond. Care was taken to prepare fresh samples since aliquots frozen with reducing agents such as DTT showed altered solubility characteristics and electrophoretic patterns[33]. The disparity in molecular weight between the subunits and native protein can be attributed to the carbohydrate moieties which affect the electrophoretic mobility of glycoproteins in SDS polyacrylamide gels[34,35]. Densitometric analysis of the purified BFA resolved on the gel (Figure III-4, page 91) indicates the protein was essentially pure (93% integration value). The antisera raised against the purified lectin gave a single precipitin line (Plate III-3 ii, page 93), evidence that the purified material contained a single lectin.

BFA is similar to the other two Moraceae lectins characterized to date (Table III-7, page 88). *Maclura pomifera* agglutinin with a molecular weight of 42,000 is a tetramer composed of subunits of 10,000 and 12,000[36]. *Artocarpus integrifolia* lectin has dissimilar subunits of 13,000 and 18,000 and an overall molecular weight of 62,000[37] although a molecular weight of 39,500 with one subunit of 10,000 has also been proposed[38]. An earlier study[39] reported subunits of 11,250 and 15,000 for the jackfruit.

Isoelectric focusing revealed that the 19,000 dalton subunit of BFA (determined by two-dimensional electrophoresis) exhibits an isolectric point of 5.2 (Plate III-6, page 96). When the isoelectric focusing gel was transferred onto a SDS polyacrylamide gel and the proteins electrophoresed in a second dimension (Plate III-7, page 97), the 22,000 subunit was located at a position corresponding to the origin of the isolectric focusing gel. The absence of the second band suggests this component possesses a sufficiently acidic pI to prevent it from entering the isoelectric focusing gel or that it forms aggregates, even in the presence of 9M urea, which are too large to penetrate the gel matrix. However, the aggregates are dissociated in SDS PAGE gels, as is evident by the presence of two bands.

The total amino acid composition of BFA was characterized by a high glycine (14.2%) and serine (11.5%) content as well as a moderately high number of glutamyl (9.3%) and aspartyl (9.0%) residues. In comparison, the levels of histidine (1.2%), arginine (2.0%) and methionine (0.7%) were relatively low (Table III-6, page 87). It is interesting to note that jackfruit lectin is high in glycine and serine and low in histidine, arginine and methionine[37] as well.

The acidic nature of BFA was also suggested in the isoelectric focusing studies described above. Despite its similarity to peanut agglutinin (PNA) in terms of T-binding specificity, BFA's amino acid composition is considerably different from that of PNA.

Among the various synthetic carbohydrate haptens used to determine the sugar binding specificity, $\beta$-D-gal(1$\rightarrow$3)$\alpha$galNAc produced the highest inhibitory activity of the

disaccharides tested (Table III-4, page 85). This specificity corresponds to the anti-T lectin from peanut[40] which has been thoroughly investigated. BFA binds the α-anomer (β-D-gal(1→3)αgalNAc) better than the β-anomer (β-D-gal(1→3)βgalNAc) and this is the inverse of that observed with peanut agglutinin[41]. However, the affinity of BFA to T-hapten gave a 50% inhibition value of 0.15 mM (81 ug/ml). BFA is paradoxical in that it has an affinity for binding the T-antigen, but will hemagglutinate erythrocytes without prior treatment with neuraminidase. From limited data (Table III-4, page 85), it appears that the lectin binds a subterminal T as well (αfuc(1→2)βgal(1→3)αgalNAc) since this compound also inhibits in this assay. The inhibition by galNAc in the free form suggests binding perhaps also involves the N-acetyl portion. In addition to BFA and PNA, *Bauhinia purpurea*, *Maclura pomifera* and *Sophora japonica* are also specific for D-galβ(1→3)DgalNAc residues[42]. The jackfruit lectin is specific for α-D-galactose[31] and N-acetyl-galactosamine[37]. An anti-T lectin in jackfruit has been described as well[43]. In view of the T-antigen binding characteristic, BFA may be potentially useful in T-antigen expressing tumor binding and biodistribution studies.

The jackfruit lectin has a number of interesting properties including the ability to agglutinate sperm[37], mitogenicity[44,45], IgA-binding[46] and enhancement of IgE synthesis through multiple oral dosing[47]. Given the taxonomic and botanical relatedness of jackfruit and breadfruit, along with the physicochemical similarities of the lectins from these species, BFA may also share some of these interesting and unique immunological properties.

Table III-1. Distribution of breadfruit agglutinin in the various parts of the plant as determined by immunodifusion analysis.

| Part | Anti-MPA[1] |
|---|---|
| Fruit | - |
| Stalk | - |
| Seed | +++ |
| Seed coat | + |
| Latex | ++ |

[1]No precipitin line (-), faint (+), moderate (++) or intense (+++)

Table III-2. Purification protocol of breadfruit agglutinin[1].

| Purification step | Total Protein (mg) | Vol (ml) | Reciprocal endpoint | Units of activity[2] | Specific activity[3] | Yield | Fold purification |
|---|---|---|---|---|---|---|---|
| crude extract | 1247 | 2710 | 1024 | $2.8 \times 10^7$ | 22.3 | - | - |
| $MnCl_2$ supernatant | 840 | 2710 | 6144 | $1.7 \times 10^7$ | 19.8 | 60 | 0.9 |
| 30% supernatant | 486 | 2860 | 2048 | $5.9 \times 10^7$ | 120.5 | 211 | 5.4 |
| 30-75% dialyzed | 189 | 215 | 131,072 | $2.8 \times 10^8$ | 1491.0 | 1016 | 67 |
| affinity purified | 10 | 25 | 131,072 | $3.3 \times 10^7$ | 3276.8 | 118 | 147 |

[2]A unit of agglutination activity represents the titratable endpoint of a given fraction using human red blood cells and doubling dilutions of the fraction. Thus for the sample showing an endpoint of 1/1024 units and volume of 2710 ml, the total units will be 2710 ml x 10 (since 100 $\mu$l used in hemagglutination assay) x 1024 = $2.8 \times 10^7$ units of activity.
[3]Specific activity is defined as the units of activity/g of protein.

[1]Flow diagram of purification shown below.

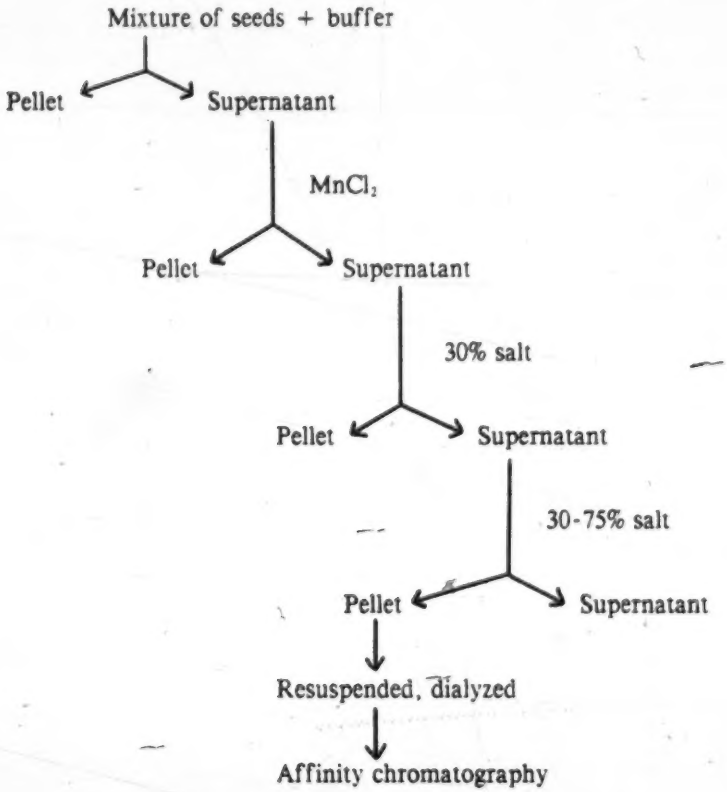

Table III-3. Agglutination of carbohydrate coated latex beads by BFA extract.

| Carbohydrate structure | Strength of agglutination |
|---|---|
| αgal(1→3)βgal(1→2)αfuc-R | + |
| αgal(1→3)βgal-R | + |
| βgal(1→4)βglc-R | + |
| βgal(1→3)βglcNAc-R | + |
| βgal-R | + |
| αgal(1→4)βgal-R | + |
| βgal(1→3)βgalNAc-R | + |
| βgal(1→4)βglcNAc(1→3)βgal(1→4)βglcNAc-R | + |
| βgal(1→4)βglcNAc-R | + + |
| βgal(1→3)βgal-R | + + |
| βgal(1→2)αman-R | + + |
| αgal-R | + + + |
| βgal(1→3)αgalNAc-R | + + + |

R = $(CH_2)_4$-$COOCH_3$

Table III-4. Inhibition of binding of T-BSA to breadfruit lectin coated tubes by carbohydrates.

| Structure | Conc. (mM) | % Inhibition |
|---|---|---|
| αgalNAc-R | 5.1 | 92.2 |
| αgal-R | 5.7 | 88.6 |
| βgal(1→3)αgalNAc-R | 3.6 | 94.1 |
| βgal(1→3)βgalNAc-R | 3.6 | 33.2 |
| αgalNAc(1→4)βgal-R | 3.6 | 32.1 |
| αman(1→2)αman-R | 3.9 | 27.7 |
| βglcNAc(1→6)αgalNAc-R | 3.6 | 25.7 |
| αgalNAc(1→3)βgal-R | 3.6 | 25.6 |
| αgal(1→3)βgal-R | 3.9 | 14.5 |
| αfuc(1→2)βgal(1→3)αgalNAc-R | 2.9 | 57.7 |
| αgalNAc(1→3)βgal(1→4)βglcNAc-R | 2.8 | 21.2 |
| αgalNAc(1→3)βgal(1→2)αfuc-R | 2.9 | 18.8 |
| βgalNAc(1→3)αgal(1→4)βgal (1→4)βglc-R | 2.3 | 19.9 |

R = $(CH_2)_1$-$COOCH_3$

Table III-5. Carbohydrate analysis of purified breadfruit lectin using the phenol-sulfuric acid method.

| BFA | ug/galactose equivalent | Percentage of carbohydrate |
|---|---|---|
| Affinity purified BFA | | |
| 60 ug | 193.61 | 209.7 |
| 150 ug | 316.94 | |
| 300 ug | 315.83 | |
| Affinity purified BFA rechromatographed[1] | | |
| 60 ug | 3.61 | 4.26 |
| 150 ug | 5.56 | |
| 300 ug | 10.00 | |

[1]On Bio-Gel P150 using 0.1M $NH_4OH$ as the elution buffer

Table III-6. Amino acid composition of the breadfruit agglutinin.

| Amino acid | Breadfruit[1] Residues/ mole protein[2] | Breadfruit[1] Percentage | Jackfruit[3] Percentage | Peanut[4] Percentage |
|---|---|---|---|---|
| Asx | 34 | 8.97 | 9.47 | 18.35 |
| Thr[5] | 30 | 7.16 | 7.94 | 9.21 |
| Ser[5] | 56 | 11.51 | 9.93 | 9.41 |
| Glx | 31 | 9.34 | 7.12 | 4.43 |
| Pro | 19 | 4.33 | 5.16 | 2.11 |
| Gly | 106 | 14.18 | 12.16 | 8.37 |
| Ala | 27 | 4.44 | 3.37 | 5.56 |
| Val | 32 | 7.44 | 8.16 | 12.44 |
| Met | 2 | 0.71 | 1.19 | |
| Ile | 23 | 6.13 | 6.39 | 6.59 |
| Leu | 22 | 5.79 | 6.02 | 4.83 |
| Tyr | 14 | 5.31 | 7.35 | 2.26 |
| Phe | 17 | 5.81 | 7.07 | 6.63 |
| His | 4 | 1.22 | 0.75 | 1.07 |
| Lys | 19 | 5.80 | 6.51 | 4.20 |
| Arg | 6 | 2.02 | 1.42 | 2.10 |

[1]Includes both subunits.
[2]Calculated on basis of molecular weight determined from gel filtration.
[3]From reference 37.
[4]Recalculated from reference 40.
[5]Extrapolated to zero time of hydrolysis.

Table III-7. Comparative properties of breadfruit (BFA), jackfruit (JFA), Osage orange (MPA) and peanut (PNA) lectins.

| Property | BFA | JFA | MPA | PNA |
|---|---|---|---|---|
| Molecular weight (daltons) | 44,500 | 62,000 (39,500)[1] | 42,000 | 110,000 |
| Subunits | 2 | 4 | 4 | 4 |
| Subunit size (daltons) | 19,000 22,000 | 13,000 18,000 (10,000)[1] | 10,000 12,000 | 27,500 |
| Carbohydrates | 4.3% | 3% | 0 | 0 |
| Blood group specificity | None | None | None | T, Tk |
| Sugar specificity | β-D-gal(1→3)αgalNAc | α-D-gal galNAc | α-D-gal galNAc | β-D-gal(1→3)D-galNAc D-gal |
| Mitogenic | | Yes | Yes | Some sources |

[1]Values reported by a different set of authors.

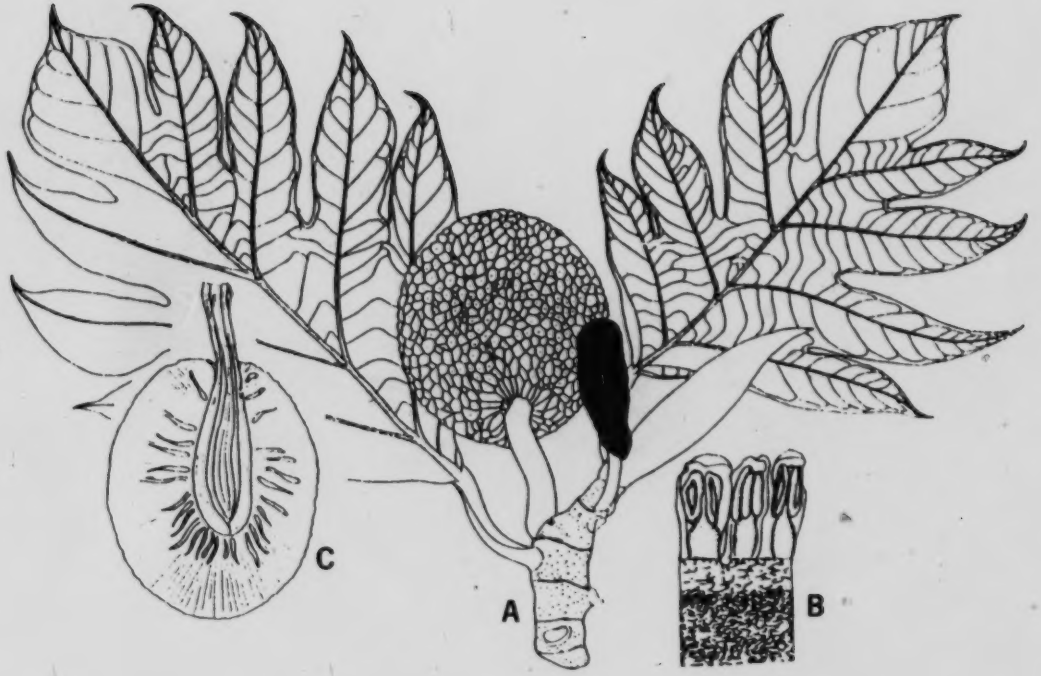


Figure III-1. Illustration of the breadfruit. A, shoot with male inflorescence and fruit; B, individual male flowers; C, fruit in longitudinal section. (Taken from Purseglove, J.W., 1968).

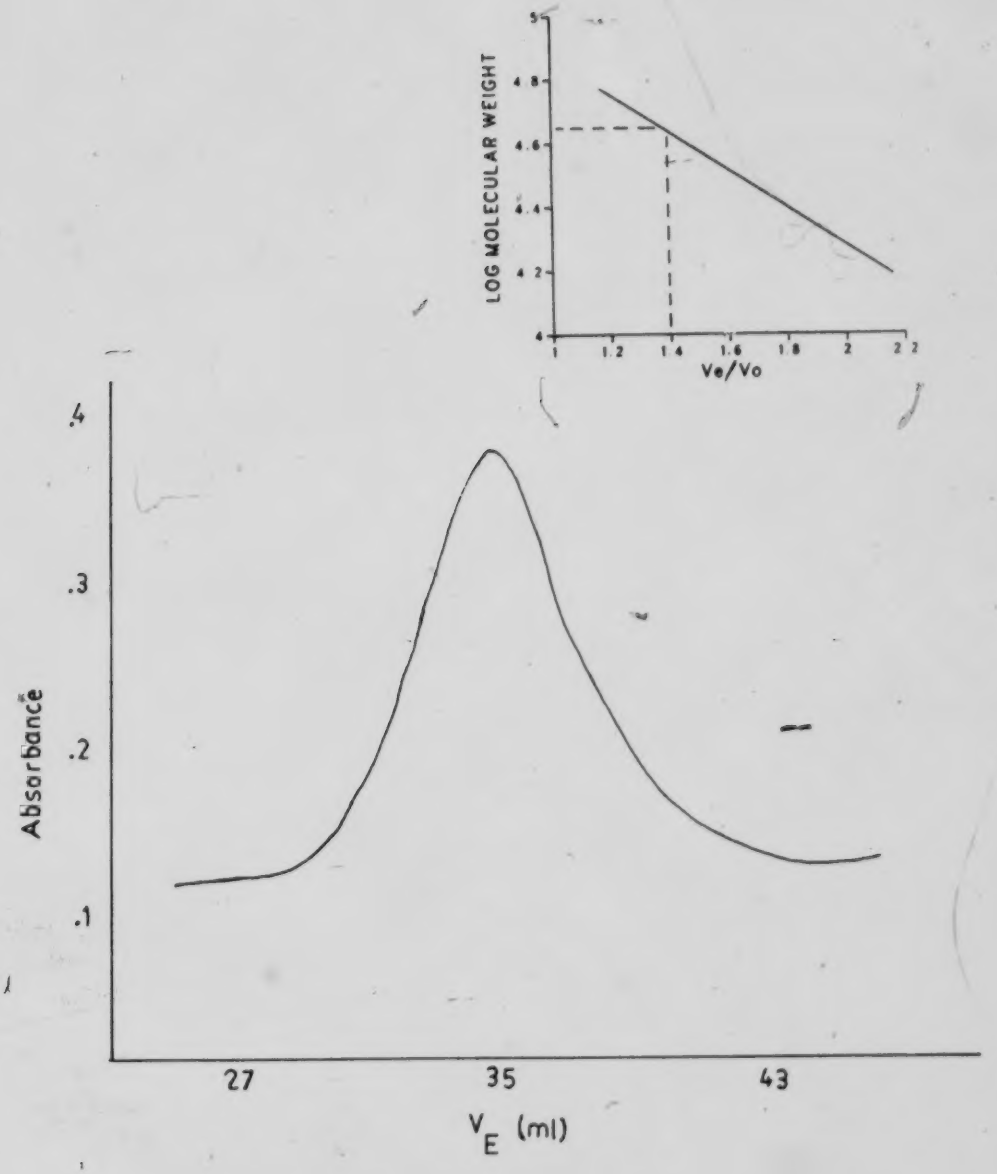


Figure III-2. Gel filtration profile of breadfruit agglutinin on Bio-Gel P150. Inset illustrates standard curve used to estimate molecular weight of the native protein.

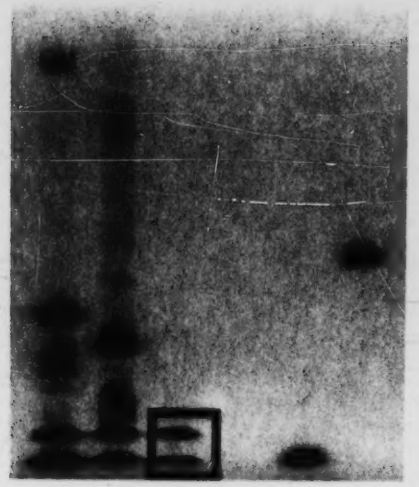

Figure III-4. Densitometric scan of purified BFA on reducing gel (boxed lane of inset).

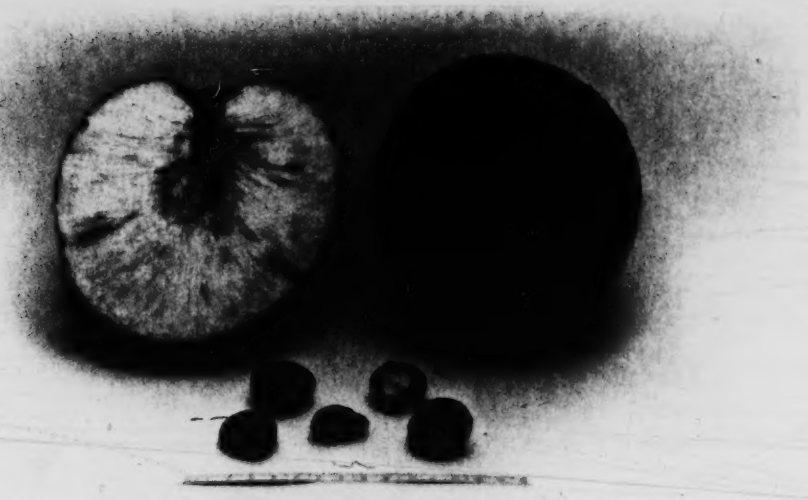

Plate III-1. An intact breadfruit (right) and a cross-section revealing the core (left). Seeds are in the foreground.

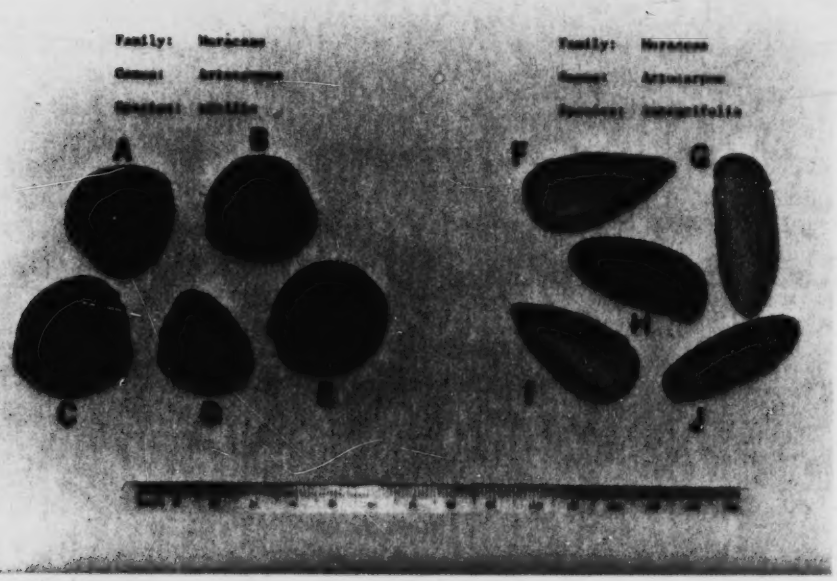


Plate III-2. Breadfruit (left) and jackfruit (right) seeds. A,E,F,G and I depict the intact seed. B and C show a cross-section of the seed. D,H and J illustrate the seed without its seed coat.

Plate III-3. i. Ouchterlony precipitin reactions of breadfruit lectin vs antisera against *Maclura pomifera* lectin. Antisera against *Maclura pomifera* (A) is surrounded by breadfruit agglutinin (1,3,5) and *Maclura pomifera* agglutinin (2,4). Note the spurs.

Plate III-3. ii. Ouchterlony precipitin reactions of *Maclura pomifera* lectin vs antisera against breadfruit lectin. Antisera against breadfruit agglutinin (B) is surrounded by preimmune sera (1), breadfruit agglutinin (2) and *Maclura pomifera* agglutinin (3).

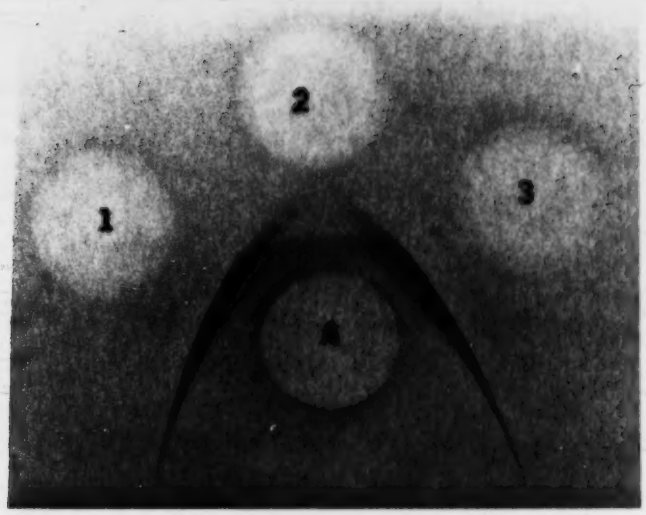


Plate III-4. Ouchterlony precipitin reaction of jackfruit extract vs antisera against *Maclura pomifera* lectin. Antisera against *Maclura pomifera* (A) is surrounded by jackfruit extract (2) and *Maclura pomifera* agglutinin (1,3).

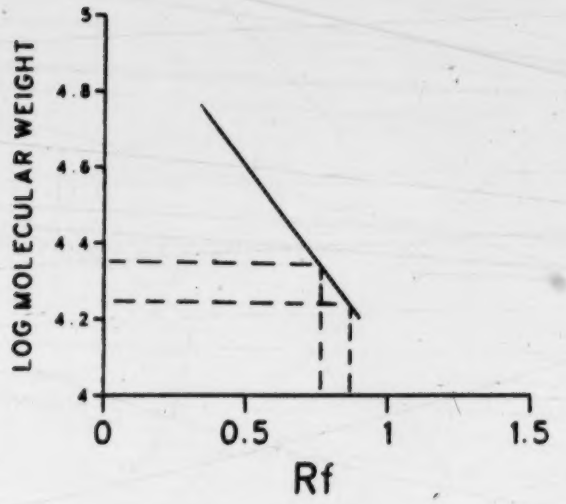


Figure III-3. The standard curve used for determining subunit molecular weights of breadfruit agglutinin.

Plate III-5. SDS polyacrylamide gel electrophoresis of breadfruit lectin on a 12% reducing gel. Lane 1, crude jackfruit; lane 2, crude breadfruit; lane 3, purified BFA; lane 4, *Machura pomifera* agglutinin; lane 5, peanut agglutinin.

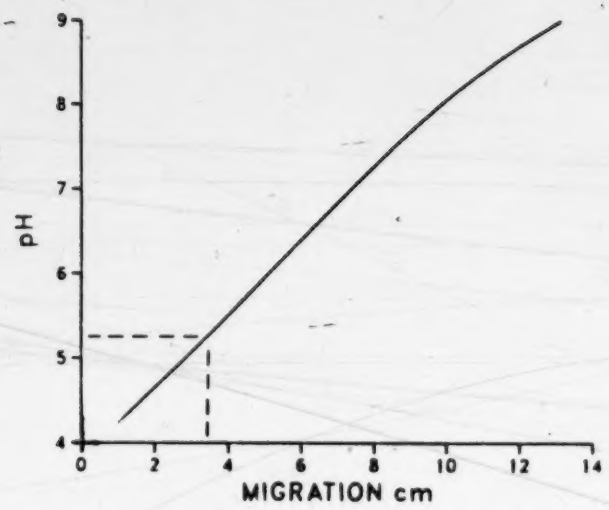


Figure III-5. The standard curve used to determine the isoelectric point of breadfruit aggulutinin.

Plate III-6. Polyacrylamide gel isoelectric focusing of breadfruit lectin.

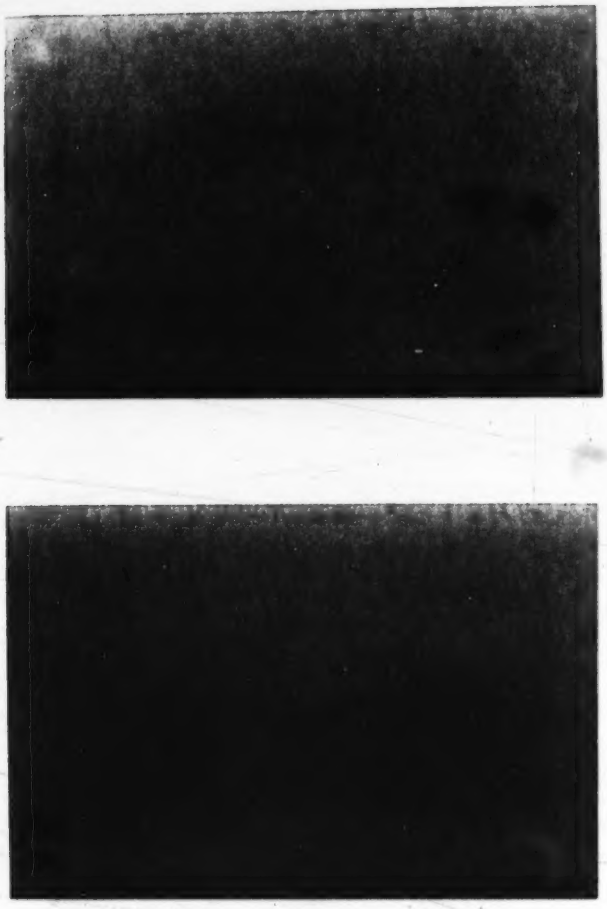

Plate III-7. Two-dimensional electrophoretic gels of crude breadfruit (top) and purified BFA (bottom). Isoelectric focusing was performed in the first dimension, followed by SDS PAGE in the second dimension.

**D. References**


1. STONE, B.C. *J. Polynes. Soc.* **83**: 92-93, 1974.

2. SMITH, A.C. Flora Vitiensis Nova - A New Flora of Fiji (Spermatocytes only) Vol.2. Angiospermae: Dicotyledons, Families 44-116. Lawai, Kauai, Hawaii: Pacific Tropical Botanical Garden, 1981.

3. BARRAU, J. *Bishop Mus. Bull.* **223**: 51-53, 1961.

4. WILDER, G.P. *Bishop Mus. Bull.* **50**: 5-83, 1928.

5. WILLIAMS, C.N. and CHEW, W.Y. Tree and Field Crops of the Wetter Regions of the Tropics. London: Longman Group Ltd., 1979.

6. COBLEY, L.S. An Introduction to the Botany of Tropical Crops. 2nd ed. Revised by W.M. Steele. New York: Longman Inc., 1976.

7. FOSBERG, F.R. *Brittonia* **12**: 101-113, 1960.

8. NA LIMA KOKUA Breadfruit (Ulu) Uses and Recipes. Lawai, Kauai, Hawaii: Pacific Tropical Botanical Garden, 1976. Revised 1977.

9. MILLER, C.D. *Bishop Mus. Bull.* **64**: 3-23, 1929.

10. REEVE, R.M. *Economic Botany* **28**: 82-96, 1974.

11. HANDY, E.S.C. *Bishop Mus. Bull.* **161**: 186-190,1940.

12. THEOBALD, W.L. *Bull. Pacif. Trop. Gard.* **6**: 1-3, 1976.

13. ALTMAN, L.J. and ZITO, S.W. *Phytochem.* **15**: 829-830, 1976.

14. PAVANASASIVAM, G. and SULTANBAWA, M.U.S. *Phytochem.* **12**: 2725-2726, 1973.

15. MAHATO, S.B., BANERJEE, S.K. and CHAKRAVARTI, R.N. *Phytochem.* **10**: 1351-1354, 1971.

16. QUIJANO, J. and ARANGO, G.J. *Economic Botany* **33**(2): 199-202, 1979.

17. GREEN, A.A. and HUGHES, W.L. *Meth. Enzymol.* **1**: 67-90, 1955.

18. PONGOR, S. and RIEDL, Z. *Anal. Biochem.* **129**: 51-56, 1983.

19. CATT, K. and TREGEAR, G.W. *Science* **158**: 1570-1572, 1967.

20. HOWARD, J. and SHANNON, L. *Anal. Biochem.* **79**: 234-239, 1977.

21. DETERMANN, H. and MICHEL, W.J. *Chromat.* **25**: 303-313, 1966.

22. LAEMMLI, U.K. *Nature (London)* **227**: 680-685, 1970.

23. O'FARRELL, P.Z., GOODMAN, H.M. and O'FARRELL, P.H. *Cell* **12**: 1133-1141, 1977.

24. FIELD, D.J. and LEE, J.C. *Anal. Biochem.* **144**: 584-592, 1985.

25. O'FARRELL, P.H. *J. Biol. Chem.* **250**(10): 4007-4021, 1975.

26. OSBORN, T.C., BROWN, J.W.S. and BLISS, F.A. *Theor. Appl. Genet.* **70**: 22-31, 1985.

27. BLACKBURN, S. Amino Acid Determination. New York: Marcel Dekker, Inc., 1968.

28. ASHWELL, G. *Meth. Enzymol.* **8**: 85-95, 1966.

29. WAXDAL, M.J. *Biochem.* **13**: 3671-3677, 1974.

30. GALBRAITH, W. and GOLDSTEIN, I.J. *Meth. Enzymol.* **28**: 318-323, 1972.

31. KUMAR, G.S., APPUKUTTAN, P.S. and BASU, D. *J. Biosci.* **4**: 257-261, 1982.

32. ANDREWS, P. *Biochem. J.* **91**: 222-232, 1964.

33. CHERRY, J.P. and ORY, R.L. *J. Agr. Food Chem.* **21**(4): 656-660, 1973.

34. SEGREST, J.P., JACKSON, R.L., ANDREWS, E.P. and MARCHESI, V.T. *Biochem. Biophys. Res. Commun.* **44**(2): 390-395, 1971.

35. BRETSCHER, M.S. *Nature New Biology* **231**: 229-232, 1971.

36. BAUSCH, J.N. and PORETZ, R.D. *Biochem.* **16**(26): 5790-5794, 1977.

37. NAMJUNTRA, P., MUANWONGYATHI, P. and CHULAVATNATOL, M. *Biochem. Biophys. Res. Commun.* **128**(2): 833-839, 1985.

38. APPUKUTTAN, P.S. and BASU, D. *FEBS Lett.* **180**(2): 331-334, 1985.

39. DE AZEVEDO, MOREIRA, R. and AINOUZ, I.L. *Plant Physiol* **61**(suppl 118), 1978.

40. LOTAN, R., SKUTELSKY, E., DANON, D. and SHARON, N. *J. Biol. Chem.* **250**: 8518-8523, 1975.

41. NOUJAIM, A.A., SHYSH, A., ZABEL, P. *et al.* In: Radioimmunoimaging and Radioimmunotherapy. Edited by S.W. Burchiel and B.A. Rhodes. Amsterdam: Elsevier, 1983.

42. WU, A.M. *Mol. Cell. Biochem.* **61**: 131-141, 1984.

43. CHATTERJEE, B.P. and UHLENBRUCK, G. *Experientia* **38**: 1225-1226, 1982.

44. BUNN-MORENO, M.M. and CAMPOS-NETO, A. *J. Immunol.* **127**(2): 427-429, 1981.

45. SOARES, M.B.M., ARMADA, J.L.A., SOARES, V.M.F.C. and SEUANEZ, H.N. *Cytobios* **34**: 165-174, 1982.

46. ROQUE-BARREIRA, M.C. and CAMPOS-NETO, A. *J. Immunol.* **134**(3): 1740-1743, 1985.

47. RESTUM-MIGUEL, N. and PROUVOST-DANON, A. *Immunology* **54**: 497-504, 1985.

## IV. PRELIMINARY INVESTIGATION OF AN AGGLUTININ FROM *FICUS DELTOIDEA* SEEDS *

### A. Introduction

Traditionally, lectins have been purified by affinity chromatography with biospecific adsorbents[1-4]. This approach necessitates a preliminary study to determine the best adsorbent. In addition, unwanted starches and sugars may also bind, making further purification necessary. Fast protein liquid chromatography (FPLC)[5] offers a fast, reproducible method for protein purification. Developed by Pharmacia, the FPLC system is a modular high performance liquid chromatography apparatus designed to purify proteins, polypeptides and polynucleotides by ion exchange chromatography, chromatofocusing, gel filtration and reverse phase chromatography. The basis of the system is a series of prepacked high resolution columns and media. FPLC has been used to purify a number of proteins such as haemoglobin derivatives[6], snake venom[7], enzymes[8] and plasma proteins[9]. The suitability of using FPLC for lectin purification is described in this chapter. Affinity purified and crude samples of breadfruit agglutinin discussed in the previous chapter were analyzed on FPLC to test the efficacy of this approach. Subsequently, *Ficus deltoidea* Jack (*F. diversifolia* Blume) or mistletoe fig[10] was chosen for FPLC purification in view of the abundance of agglutinating material and bacterial agglutinations observed in preliminary screening experiments.

### B. Materials and Methods

1) Materials

*F. deltoidea* seeds were obtained from plants growing in the Department of Plant Science, University of Alberta. Chembiomed Ltd. (Edmonton) provided carbohydrate-bound latexes. The FPLC equipment and column were bought from Pharmacia Fine Chemicals (Uppsala, Sweden).

---

**A version of this chapter has been published in Biochemical Archives, 2(2): 99-105, 1986.*

2) Preparation of crude seed extracts

Since the seeds are very small, the entire fruit and seeds were used. One gram of fruit and seeds (Plates IV-1, 2, page 106) was ground using mortar and pestle, mixed with 10 ml 0.01M PBS and tumbled overnight at 4°C. After centrifuging for 15 min. at 320 *x g* in a Dynac table top centrifuge, the supernatants were collected and centrifuged again under the same parameters. Extracts were stored at -20°C and then freeze-dried.

3) Latex screening for affinity purification

The extract was screened against a series of carbohydrate-bound latexes (developed by Chembiomed Ltd. and described in reference 11) to give an indication of the sugar specificity.

4) Fast protein liquid chromatographic purification of FDA and BFA

Crude seed extracts of *F.* deltoidea and *A. altilis* and the affinity purified BFA were chromatographed on a Pharmacia HR 10/30 Superose 12™ high performance gel filtration column, an agarose-based matrix in a prepacked glass column[12-13] with a fractionation range of 1,000-300,000 daltons. The Pharmacia FPLC system equipped with the Liquid Chromatography Controller (LCC), 280 nm UV monitor, P-500 high precision pump and Frac-100 fraction collector was utilized. Samples (200 ul of a 1.5 mg/0.5 ml solution) were loaded on the column and sodium acetate buffer (0.05M, pH 5.2) containing 0.1M NaCl was used as an eluant with a flow rate of 0.3 ml/min. The peak fractions were collected automatically in 5 ml tubes using the peak collection mode, dialyzed against 2L of distilled water overnight and tested for hemagglutination activity.

5) Screening assays

Hemagglutination and Ouchterlony assays and bacterial agglutinations were performed as described in chapters II and III.

**C. Results and Discussion**

Titers ranging from 1/6400 to 1/8200 found in hemagglutination screening studies (chapter II) indicated an abundance of agglutinating material present in the crude fig preparation. Antisera directed against *M. pomifera*, *A. hypogaea*, *T. vulgaris*, *D. biflorus*, *C. ensiformis*, *L. polyphemus*, *T. purpureas* and *P. sativum* agglutinins showed a precipitin line with the *F. deltoidea* extract using Ouchterlony immunodiffusion assays (Plate IV-3, page 107), despite the presence of various sugars in the agar to eliminate spurious interactions. It is difficult to justify a lectin cross-reacting with antisera of so many phylogenetically different and varied lectins. The lectins screened above belong to such diverse taxa as Moraceae, Leguminosae, Gramineae and even an invertebrate. Further, as already alluded to in chapter II, the fig extract also agglutinated various broths. These apparently interesting features were pursued to determine the nature of the active principle. When the crude FDA extract was mixed with normal rabbit sera, the precipitin reactions obtained with various antisera were eliminated suggesting the lectin is interacting with a component in sera to form a precipitin line. It is pertinent to note at this juncture, that extracts of certain bacteria such as *Staphylococcus aureus* possess or exhibit immunoglobulin binding properties. This protein (protein A) has been purified and extensively characterized and is routinely used as an important probe in immunology. Among all the sugars tested, it should be emphasized that free sialic acid and latex-bound sialic acid were omitted due to lack of availability. Sialic acid binding lectins are rare but known to exist, an example being the lectin from the invertebrate *Limulus polyphemus*.

FDA is also of interest for its ability to agglutinate some strains of bacteria, including *S. aureus*, *B. cereus* and *Chlamydia trachomatis*, the latter being a clinically significant pathogen responsible for a venereal disease. Chlamydial attachment to mouse fibroblasts (L cells) has been blocked using wheat germ agglutinin[14]. Other studies using this lectin as a probe[15-16] suggest the attachment of *C. trachomatis* is related to $\beta 1 \rightarrow 4$-linked oligomers of D-glcNAc and NeuNAc residues.

No agglutination was observed between different latexes and the FDA extract, making the affinity separation of the extract more difficult since a suitable adsorbent could not be readily identified. Attempts were made to purify the agglutinin from FDA using a novel approach. In order to validate the FPLC system, crude and affinity purified samples of BFA described in the previous chapter were chromatographed to determine the feasibility for lectin purification. Application of crude BFA to a Superose 12™ gel filtration column produced several peaks. Erythrocyte agglutination activity was exclusively associated to the peak which eluted at 20.15 min. Gel filtration of the affinity purified BFA produced a peak at a similar elution volume as compared to the active fraction of the crude extract (Figure IV-1 A and B, page 105). This demonstrates that gel filtration FPLC is very efficient and can eliminate the lengthy procedure of latex screening and affinity chromatography, rendering an enriched product in a very short time. Gel filtration of the crude FDA extract resulted in nine major peaks (Figure IV-2, page 105). When tested, all of the peaks agglutinated erythrocytes but only the fourth peak was active against the bacterium *Chlamydia*. Thus, by the use of gel filtration FPLC, the crude FDA was separated into nine components in less than 20 min. and only one fraction agglutinated *Chlamydia*. Evidently, gel filtration FPLC may be used in the purification of lectins from different sources and is an alternative to the traditional affinity chromatograpy methods because it is simple, fast and reproducible.

FDA has proved to be an interesting lectin since it can agglutinate *Chlamydia* as well as precipitate certain serum components as noted earlier. The same principle or two different ones may be involved. These properties may be useful in developing a rapid means of *Chlamydia* identification or as a probe for membrane studies of the bacterium.

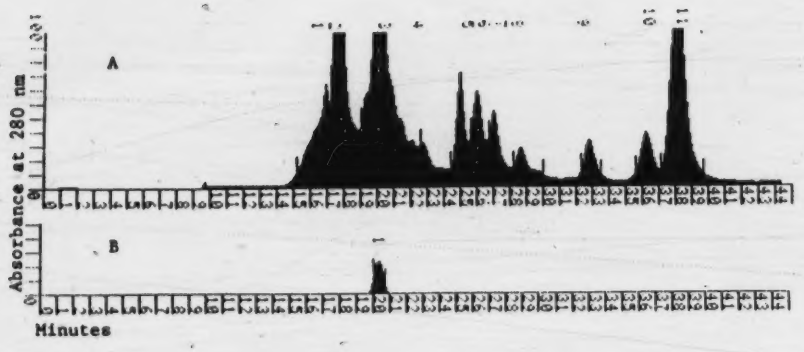


Figure IV-1. Gel filtration profile of crude (A) and affinity purified (B) BFA extracts on Superose 12™. Peaks 3 in A and 1 in B represent the active fractions.

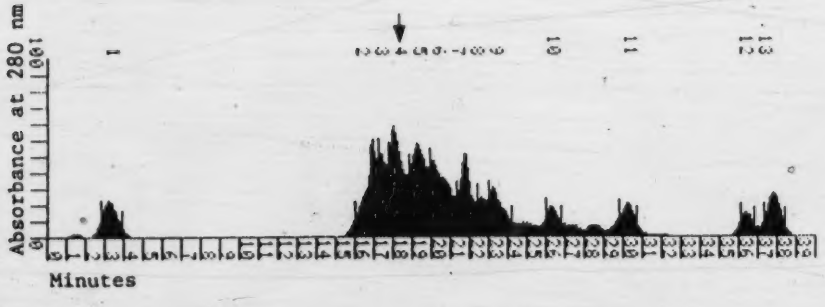


Figure IV-2. Gel filtration profile of FDA crude extract on Superose 12™. Peak 4 represents the active fraction.

Plate IV-1. A branch of *Ficus deltoidea* bush showing leaves and fruit.

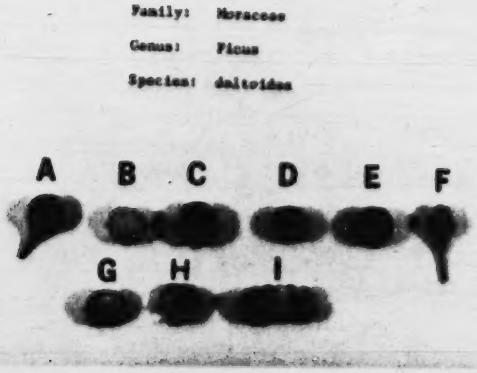


Plate IV-2. Fruit and seeds of *Ficus deltoidea*. A to F illustrate stages of ripening. G to I depict a cross-section of the fruit revealing the seeds.

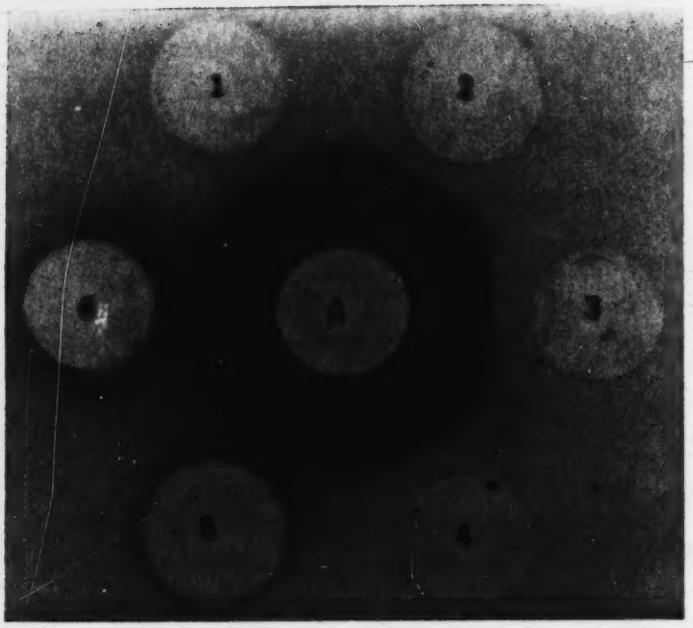


Plate IV-3. Ouchterlony immunodiffusion assay of *F. deltoidea* extract (A) surrounded by antisera directed against *T. purpureas* (1), *D. biflorus* (2), *A. hypogaea* (3), *M. pomifera* (4), *C. ensiformis* (5) and *L. polyphemus* (6) agglutinins. Similar reactions were observed with *P. sativum* and *T. vulgaris*.

**D. References**


1. BESSELER, W. and GOLDSTEIN, I.J. *FEBS Lett.* **34**(1): 58-62, 1973.

2. LIS, H., LOTAN, R. and SHARON, N. *Ann N.Y. Acad. Sci.* **234**: 232-238, 1974.

3. VRETBLAD, P. *Biochim. Biophys. Acta* **434**: 169-176, 1976.

4. LIS, H. and SHARON, N. *J. Chromat.* **215**: 361-372, 1981.

5. RICHEY, J. *Amer. Lab.* **14**(10): 104-129, 1982.

6. KRAMLOVA, M., PRISTOUPIL, T.I., FRICOVA, V., KRAML, J., BERGLUND, R. and KANTARDJIEV, N. *J. Chromat.* **249**: 403-404, 1982.

7. DYR, J.E., FORTOVA, H., VODRAZKA, Z. and KORNALIK, F. *J. Chromat.* **261**: 163-165, 1983.

8. MARKEY, F. *FEBS Lett.* **167**: 155-159, 1984.

9. COOPER, E.H., TURNER, R., JOHNS, E.A., LINDBLOM, H. and BRITTON, V.J. *Clin. Chem* **29**: 1635-1640, 1983.

10. CORNER, E.J.H. *Roy. Soc. Lond. Phil. Trans. B*, **256**: 281-317, 1969.

11. PONGOR, S. and RIEDL, Z. *Anal. Biochem* **129**: 51-56, 1983.

12. HAGEL, L. and ANDERSSON, T. *J. Chromat.* **285**: 295-306, 1984.

13. ANDERSSON, T. and HAGEL, L. *Anal. Biochem.* **141**: 461-465, 1984.

14. LEVY, N.J. *Infect. Immun.* **25**(3): 946-953, 1979.

15. SODERLUND, G. and KIHLSTROM, E. *Infect Immun.* **42**(3): 930-935, 1983.

16. BOSE, S.K., SMITH, G.B. and PAUL, R.G. *Infect Immun.* **40**(3): 1060-1067, 1983

## V. GENERAL SUMMARY AND CONCLUSIONS

The screening of 23 plant species using hemagglutination and Ouchterlony immunodiffusion assays revealed high titers of agglutinating material in six species, low titers in nine and no agglutination in eight. The agglutination was observed with A, B, O and AB classes of human erythrocytes in all cases. The two *Artocarpus* species (*A. integrifolia*, *A. altilis*) showed reactions of partial identity with antisera directed against *Maclura pomifera* agglutinin, another member of the Moraceae family. The cross-reactivity between the *Artocarpus* and *Maclura* lectins suggests a phylogenetic relationship between lectins of the Moraceae since all three belong to this taxon. Moraceae lectins thus appear to be related and exhibit considerable conserved domains in the protein structure.

An extract from *Ficus deltoidea* reacted with all eight antisera tested. These precipitin reactions were eliminated by mixing normal rabbit sera with the crude *F. deltoidea* extract, suggesting the active principle is precipitating some component in the serum. It would be interesting to pursue this further. In testing Moraceae species for bacterial agglutination, *F. deltoidea* proved interesting in agglutinating some bacteria, including *Chlamydia*. *Broussonetia papyrifera* lectin slightly agglutinated *Bacillus cereus* as well as *Chlamydia*. The remaining Moraceae extracts tested failed to agglutinate the different species of bacteria used in this study.

On the basis of the aforementioned results, *Artocarpus altilis* (breadfruit) and *Ficus deltoidea* (mistletoe fig) were selected for further investigation. The breadfruit agglutinin was purified by steps involving salt fractionation and affinity chromatography on immobilized $\beta$-D-gal(1→3)$\alpha$galNAc. Characterization of the purified material revealed a molecular weight of 44,500 for the native protein, consisting of two dissimilar subunits of approximately 19,000 and 22,000 daltons. The carbohydrate content was estimated to be 4.3%. The amino acid composition was noteworthy for a high content of glycine and serine and moderate amounts of glutamyl and aspartyl residues. The levels of histidine, arginine and methionine were

relatively low. $\beta$-gal(1→3)$\alpha$galNAc produced the highest inhibitory activity of the disaccharides tested. In view of this T antigen binding characteristic of the breadfruit agglutinin, tumor binding studies are proposed as an extension of this work.

The traditional approach to lectin purification involving affinity chromatography with biospecific adsorbents requires much ground work. Consequently, a novel approach to the purification of the lectins was undertaken. The viability of utilizing fast protein liquid chromatography was initially demonstrated with crude extracts as well as affinity purified samples of breadfruit agglutinin, which was purified by the conventional method noted above. FPLC offers the advantages of being fast, simple and highly reproducible. This procedure resolved the *F. deltoidea* extract into nine major peaks, all of which agglutinated human erythrocytes. Purified elementary bodies of the bacterium *Chlamydia* were only agglutinated by material in the fourth fraction. Thus, purification may be accomplished by means other than the conventional affinity chromatography. The serum binding characteristic of *F. deltoidea* agglutinin also merits further investigation. Given the importance of *Chlamydia* as a clinically significant pathogen, FDA may have value as a probe for studying and identifying this pathogen.

## VI. APPENDIX

### Seed Sources

Hemp

Bureau of Dangerous Drugs,
Health Protection Branch,
Department of National Health and Welfare,
3rd floor, Jackson Building,
122 Bank St.,
Ottawa, Ont. K1A 1B9

Douglas fir, white spruce, lodgepole pine, Siberian larch

Alberta Agriculture,
Alberta Tree Nursery and Horticulture Centre,
R.R. 6,
Edmonton, Alta. T5B 4K3

Polish rapeseed, Argentina rapeseed

United Grain Growers,
Seed Division,
7410 - 120 Ave.,
Edmonton, Alta.

Black manh, moth, red chori

Asia Import House (1980) Ltd.,
7912 - 104 St.,
Edmonton, Alta.

Paper mulberry, Osage orange

F.W. Schumacher Inc.,
Sandwich, Mass.
02563-1023
U.S.A.

Breadfruit, custard apple

JB Q-Mart,
8929 - 82 Ave.,
Edmonton, Alta.

Common fig, Japanese hop

J.L. Hudson, Seedsman,
P.0. Box 1058,
Redwood City, California
94064
U.S.A.

Jackfruit

Dr. A. Srinivasan,
Director, Protein Research Unit,
Department of Chemistry,
Loyola College,
Madras,
South India

Dr. C.K. Rao
Department of Botany,
Bangalore University,
Jnanabharathi,
Bangalore 560056
India

Lychee, persimmon, McIntosh apple, cherimoya, mango

Safeway Stores Ltd.,
Edmonton, Alta.

Mistletoe fig, American tropical fig

Mr. R. Kroon,
Greenhouse supervisor,
Department of Botany,
University of Alberta,
Edmonton, Alta.

Mr. H. Welling,
Greenhouse supervisor,
Department of Plant Science,
University of Alberta,
Edmonton, Alta.

## VII. CURRICULUM VITAE

NAME: John Bradley Hunter

PLACE OF BIRTH: London, Ontario, Canada

DATE OF BIRTH: November 19, 1953

POST-SECONDARY EDUCATION:

Hon. B.A. (Philosophy)
University of Western Ontario
London, Ont.
June 1975

M.A. (Journalism)
University of Western Ontario
London, Ont.
October 1976

Hon. B.Sc. (Microbiology & Immunology)
University of Western Ontario
London, Ont.
June 1983

M.Sc. (Pharmaceutical Sciences)
(Awarded in part for this thesis)
University of Alberta
Edmonton, Alta.
November 1986

HONOURS AND AWARDS:

C.H. Ivey Foundation Bursary, 1972-75

Dean's Honour List, 1974-75

Alberta Heritage Foundation for Medical Research
Studentship, 1984-86

RELATED WORK EXPERIENCE:

Teaching assistant, Faculty of Pharmacy &
Pharmaceutical Sciences,
University of Alberta, 1983-86

Research assistant, department of
microbiology & immunology,
University of Western Ontario, summer 1983

Data analyst, department of physiology,
University of Western Ontario, summer 1982

Assistant editor, Business Publications Div.,
Maclean-Hunter Ltd.,
Toronto, Ont., 1976-79

Research assistant, School of Journalism,
University of Western Ontario, 1975-76

PUBLICATIONS:

Hunter, J.B. *et al*. Purification of Lectins from *Artocarpus altilis* and *Ficus deltoidea* by Gel Filtration Fast Protein Liquid Chromatography. *Biochemical Archives*, 2(2): 99-105, 1986.

Hunter, J.B. *et al*. Isolation and Characterization of a Lectin from Breadfruit (*Artocarpus altilis*) Seeds. *Biochemical Archives*, 1986 (in press).

Hunter, J.B. *et al*. Physicochemical Properties of Lectins - A Review. In preparation.